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Myeloid-derived suppressor cells: Natural regulators for transplant tolerance

Peter Boros[a,*], **Jordi C. Ochando**[b], **Shu-Hsia Chen**[b], and **Jonathan S. Bromberg**[a,b]

[a]Recanati/Miller Transplantation Institute, Mount Sinai School of Medicine, New York, New York, USA

[b]Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, New York, USA

Abstract

Myeloid derived suppressor cells (MDSC) contribute to the negative regulation of immune response in cancer patients. This review summarizes results on important issues related to MDSC biology, including expansion and activation of MDSC, phenotype, and subsets as well pathways and different mechanisms by which these cells exert their suppressive effect. Recent observations suggesting that MDSC may have roles in transplant tolerance are presented. Although therapeutic targeting and destruction of MDCS is of primary interest in cancer patients, in transplantation it will instead be necessary to induce, expand, and activate these cells; thus current possibilities for *in vitro* generation of MDSC are also discussed.

Keywords

Myeloid derived suppressor cells; Organ transplantation; Tolerance

1. Introduction

Myeloid-derived suppressor cells (MDSC) were originally described more than 20 years ago in cancer patients, and it was suggested that MDSC might contribute to the negative regulation of immune response to tumors. MDSC were defined as a morphologically and functionally heterogeneous population consisting of progenitor cells of macrophages, granulocytes, dendritic cells (DC), and immature myeloid cells (IMC). Normally, IMC migrate to different peripheral organs and differentiate into macrophages, DC, or granulocytes [1]. In the tumor microenvironment, several factors support the accumulation of IMC, prevent their differentiation, and induce their suppressive function [2,3]. Indeed, large numbers of MDSC amass in lymphoid tissues of tumor-bearing mice. Up to 40% of nucleated splenocytes are MDSC in tumor bearing mice, compared with 5% in normal animals; and MDSC are found in tumor tissues as well as in the lymph nodes [4,5]. Similarly, the numbers of circulating MDSC significantly increase in cancer patients compared with healthy individuals, and seem to correlate with the clinical stage [6]. The challenges remain to distinguish phenotypically the heterogeneous populations of MDSC, understand their lineage commitments and developmental pathways, and define the signals that induce their maturation.

*Corresponding author: peter.boros@mssm.edu (P. Boros).

Changes in MDSC numbers are not limited to reactions to different tumors. Their accumulation has also been detected in mice with various acute and chronic infectious diseases, graft-versus-host disease, sepsis, and immune stress after activation by superantigens and trauma. Evidence suggests that the expansion of these regulatory cells may represent a common response to all forms of inflammation.

MDSC have been associated with many diverse regulatory functions, including tumor-associated immune defects, suppression of T-cell responses related to adaptive immune response in both antigen-specific and non–antigen-specific manners depending on the conditions of T-cell activation, and regulation of the innate immune response. Recently, MDSC have been considered a possible target for therapeutic intervention [7,8]. In this review, we aim to summarize knowledge on several important issues related to MDSC biology and the possible role of these cells in organ transplantation.

2. Expansion and activation of MDSC are associated with diverse pathologic conditions

MDSC expand systemically both in mice inoculated with tumor cells and in animals developing spontaneous malignancies. A marked increase in MDSC numbers is also detected in the blood of patients with many different types of cancers. Accumulation of MDSC in lymphoid organs and in blood is associated with several infectious conditions, including mice infected with *Mycobacterium tuberculosis*, *Trypanosoma cruzi*, *Toxoplasma*, *Listeria monocytogenes*, *Leishmania major,* and *Candida albicans* [9–12]. MDSC expansion is associated with autoimmunity, inflammation, and traumatic stress as shown by experimental models of autoimmune uveoretinitis, autoimmune encephalitis, and inflammatory bowel disease [13–15]. Considerable increases in MDSC numbers are observed in normal mice after immunization with ovalbumin or peptides [16].

The expansion and activation of MDSC are regulated by factors produced by tumor cells, activated T cells, and stromal cells. There are partially overlapping activities of these factors, which may allow for flexibility in the regulation under physiologic and pathologic conditions. Expansion inducing factors include macophage–colony stimulating factor(CSF), granulocte-macophage-CSF, vascular endothelial growth factor (VEGF), stem cell factor, interleukin (IL)–6, and prostaglandins and their regulator, cyclooxygenase (COX)–2 [17–20]. These factors exert their effects by stimulating myelopoiesis and by inhibiting differentiation of mature myeloid cells. They trigger the JAK1 and STAT3 signaling pathways involved in cell survival, proliferation, and differentiation [21,22]. STAT3 activation is associated with increased survival and expansion of myeloid progenitor cells. Selective STAT3 inhibitors reduced the expansion of MDSC, while increasing T-cell responses in tumor bearing mice, suggesting a central role for this signaling pathway in MDSC expansion [23]. STAT3 activation upregulated the expression of calcium-binding proteins S100A8 and S100A9. S100A8 and A9 are proteins with diverse functions regulating cell migration, cytoskeletal–membrane interactions, neutrophil activation, and kinase activities. They influence leukocyte transmigration into tissues by increasing leukocyte deformability and integrin-mediated adhesion. Increased expression of these proteins in MDSC prevents differentiation and promotes expansion [24].

3. Phenotype and subsets

MDSC detected in pathologic conditions are a heterogeneous population of activated IMC that have been prevented from fully differentiating into mature cells. Approximately 1%–5% of MDSC can form myeloid cell colonies, and one third of this population can differentiate into mature macrophages and DC in an appropriate cytokine milieu [25].

In tumor-bearing mice, these cells are defined as Gr-1$^+$ CD11b$^+$ (αM-integrin) cells co-expressing the immature cell marker CD31. From 20% to 30% of bone marrow cells display this phenotype. The cells are absent from the lymph nodes, whereas the spleen has 5–7%. Additional markers, possibly related to suppressive function, include CD80, F4/80, CD40, CD115 (macrophage colony-stimulating factor receptor), CD124 (IL-4Rα) and CD16 (FcγRIII) [4,5,26–28]. MDSC express MHC class I but low class II molecules. Based on their expression of Gr-Gr-1, morphologic heterogeneity of MDSC has been further defined in mice. Gr-1–specific antibodies may bind to two epitopes, Ly6G and Ly6C. Epitope-specific antibodies identify two murine MDSC subsets: granulocytic MDSC have a CD11b$^+$ Ly6G$^+$ Ly6C^{low} phenotype, whereas monocytic MDSC are described as CD11b$^+$ Ly6G$^-$ Ly6C^{high}. The two subsets have different characteristics in terms of their ability to differentiate, so that only the monocytic MDSC can mature into DC and macrophages. The subsets also may use different pathways to suppress T-cell responses [29].

Another study identified three subsets distinguishable by the level of Gr-1 expression: high, intermediate, and low. Morphology of CD11b$^+$Gr-1^{low} cells suggests that they represent the monocytic subset, whereas CD11b$^+$ Gr-1^{int} cells are mostly granulocytes, and the Gr-1 high population consists of mature neutrophils. This study, performed in tumor-bearing mice, failed to identify a correlation between suppressive activity and expression levels of CD115, CD124, CD80, PD-L1, and PD-L2, as cells isolated from tumor-free mice had similar expression patterns [30]. These findings underline the phenotypic heterogeneity of MDSC. The major difficulty throughout the field of MDSC research is that although these cells have a common biologic activity of suppression, phenotypically they are an extremely heterogeneous group of myeloid cells. The lineages and development of MDSC are not defined, and some reports have failed to define phenotype or even precise gating or isolation procedures. It is likely that the suppressive activity associated with MDSC represents a special functional state of the entire population of cells and is not the result of the expansion of a single well-defined subset [31,32]. A recent study using a murine model of inflammatory bowel disease and tumor-bearing mice established an important role for CD49d (an integrin α-subunit making up half of the $\alpha4\beta1$ lymphocyte homing receptor) as an alternative marker for Gr-1 to differentiate between the subpopulations of MDSC together with CD11b. Based on CD49d expression, there are two distinct subpopulations among CD11b$^+$ Gr-1dull/int MDSC. The CD49d$^+$ subset is monocytic, and the CD49d$^-$ cells are granulocytic. Functionally, the monocytic cells strongly suppress Ag-specific T-cell proliferation in a nitric oxide (NO)–dependent mechanism, whereas the CD49b$^-$ cells only poorly inhibit T-cell proliferation [33]. Human monocytic MDSC are defined as CD33$^+$/CD14$^+$/HLA-DR low [34–36], and granulocytic MDSC as CD14$^-$ CD11b$^+$ cells that express immature markers, such as CD34, CD33, CD15, and CD13, and that lack the expression of markers of mature myeloid and lymphoid cells and have low major histocompatibility complex (MHC) class II [37–40]. Although barely detectable in the peripheral blood of healthy individuals, the number of circulating MDSC is increased in cancer patients. Human cells do not have a marker homologous to mouse Gr-1 [41] Table 1).

4. Suppressor function

MDSC suppress T, B, and NK proliferation and cytokine production. Several factors induce suppressive activity in MDSC, including IFN-γ, Toll-like receptor (TLR) ligands, transforming growth factor–β, IL-4, and IL-13, which activate signaling pathways, including STAT6, STAT1 and NF-κB. STAT1 is the transcriptional pathway for IFN-γ-mediated signaling, and is involved in the upregulation of arginase 1 (Arg-1) and inducible NO synthase (iNOS) expression. Blockade of IFNγ reduces MDSC-mediated T-cell suppression. MDSC isolated from STAT1−/− mice fail to upregulate the expression of Arg-1 and iNOS [42].

Activation of the IL-4Rα signaling pathway induces Arg-1 expression in MDCS, so that both IL-4 and IL-13 upregulate the activity of Arg-1 [43]. STAT6 deficiency prevents signaling by IL-4Rα and blocks the production of Arg-1 [44]. The IL-4Rα–STAT6 pathway was involved in the IL-13-induced production of TGF-β by MDSC [45]. In murine models of sepsis, splenic expansion of MDSC depends on the TLR adapter molecule myeloid differentiation primary-response gene 88 (MyD88). As expansion of MDSC can be detected in mice lacking a functional TLR4 protein, MyD88-dependent signaling pathways triggered by other TLR contribute to the expansion of MDSC [46].

A basic mechanism by which activated MDSC suppress immune responses is through the activity of iNOS and Arg-1 [47]. iNOS induces NO production, whereas Arg-1 depletes arginine. Activation of Arg-1 and iNOS results in the suppression of T-cell responses. Depletion of L-arginine inhibits T-cell proliferation via several mechanisms, including decreasing the expression of the CD3 ζ-chain and preventing the expression of the cell cycle regulators cyclin D3 and cyclin-dependent kinase 4 [48]. NO suppresses T-cell responses by inhibiting JAK3 and STAT5 function and MHC class II expression. NO also induces T-cell apoptosis. The pivotal role of these enzymes is suggested by observations demonstrating that inhibitors of iNOS and Arg-1, such as phosphodiesterase-5 inhibitors or N^G-mono-methyl-L-arginine, reduce MDSC expansion and reverse T-cell inhibitory effects [49].

Increased production of reactive oxygen species (ROS) also contributes to the suppressive activity of MDSC. Substantial production of H_2O_2 by MDSC both in tumor models and in patients with malignancies has been reported [50,51]. The increased production was mediated by upregulated activity of NADPH oxidase (NOX2) resulting from significantly higher expression of NOX2 subunits, primarily $p47^{phox}$ and $gp91^{phox}$, compared with cells from tumor-free mice. STAT3 controls the expression of NOX2 subunits, and absence of NOX2 activity results in loss of suppressive activity. Inhibition of ROS production with the STAT3 inhibitor JSI-124 abolished MDSC-suppressive capacity in cells isolated from both mice and cancer patients [22].

Peroxynitrite, a powerful oxidant resulting from the reaction between NO and superoxide anion, induces the nitration and nitrosylation of several amino acids. High levels of peroxynitrite were detected at the site of MDSC accumulation, and were associated with tumor progression and T-cell unresponsiveness [52]. Peroxynitrite production by MDSC occurs during direct contact with T cells and leads to nitration of the T-cell receptor and CD8 molecules, preventing specific antigen peptide binding. T cells become unresponsive to antigen-specific stimulation while maintaining non-specific responsiveness [53].

The granulocytic and monocytic subsets of MDSC have differences in their functional pathways. Although both subsets express Arg-1, granulocytic MDSC express high levels of ROS and low levels of NO, whereas low levels of ROS and high levels of NO characterize the monocytic subset. All MDSC may suppress antigen-specific T-cell proliferation, yet use different effector molecules and signaling pathways. In a tumor model, the suppressive activity of the granulocytic subset depended on Arg-1, whereas the monocytic subset was dependent on STAT1 and iNOS. Similar findings were reported during *Trypanosoma cruzi* infection [54,55].

MDSC can downregulate T-cell proliferation in other ways. One mechanism is the recently identified ability of MDSC to promote the development of $Foxp3^+$ regulatory T (Treg) cells. The induction of Treg required both interferon (IFN)–γ and IL-10, but was independent of the production of NO. The CTLA4 (CD152) was also required. Injection of anti–CTLA-4 antibodies, which blocked the interactions between Treg and MDSC, into tumor-bearing mice lead to inhibition of tumor growth [15,56]. Interaction between MDSC and

macrophages resulted in a shift toward a type 2 macrophage responses, with reduced IL-12 release by macrophages and increased IL-10 production by MDSC, promoting tumor immune evasion [4].

Interaction between NK cells and MDSC has also been reported. The function of NK cells isolated from liver and spleen of tumor-bearing mice was impaired in all models. In orthotopic liver cancer–bearing mice, downregulation of NK cell function was shown by decreased cytotoxicity, NKG2D expression, and IFN-γ production both *in vitro* and *in vivo*, and was correlated with an increase of MDSC in liver and spleen. The function of hepatic natural killer (NK) cells was restored by MDSC, but not regulatory T-cell, depletion. MDSC isolated from patients with hepatocellular carcinoma inhibited autologous NK cell cytotoxicity and cytokine secretion in culture. Suppression was dependent on cell contact and expression of NKp30, but not Arg-1 [57,58] (Table 2).

5. MDSC and transplantation tolerance

The role of MDSC in organ transplantation tolerance was first described in a rat model of kidney allografting, in which tolerance was induced across a full MHC mismatch with anti-CD28 antibodies. $CD3^{-}$ class II^{-} $CD11b^{+}$ $CD80/86^{+}$ plastic-adherent cells accumulated in the blood of tolerant recipients, and by immunohistology cells with similar phenotype were detected in the graft. These cells were identified as MDSC that inhibited the proliferation of effector T cells, and induced apoptosis in a contact-dependent manner. Messenger RNA for iNOS was increased by several-fold in MDSC. The suppressive function was dependent on iNOS detectable in isolated MDSC as well as in graft-infiltrating cells, and injection of the iNOS inhibitor amino guanidine induced the rejection of accepted allografts. These observations suggest that, similar to tumor-infiltrating MDSC, blood-derived MDSC suppress T cells inside the graft. MDSC were also present in isograft recipients and in naive animals and had similar activity to MDSC in tolerant recipients. However, there was a significant increase in the numbers of these cells in tolerant recipients. MDSC did not affect the proliferation of $CD4^{+}CD25^{high}FoxP3^{+}$ Treg; and Treg, contrary to effector T cells, failed to induce iNOS. This suggests that MDSC might mediate suppression of the allogeneic response partly through induction or sparing of Treg [59,60].

Different pathways for MDSC suppressive activity, possibly important in transplant settings, have been reported. Exposure of mice to repeated injections of bacterial LPS was sufficient to induce MDSC. The expanded $CD11b^{+}$ $Gr\text{-}1^{+}$ cells suppressed T-cell proliferation and Th1 and Th2 cytokine production in *in vitro* assays of allogeneic responses. Transfer of MDSC into naive recipients resulted in prolongation of skin allograft survival. This study identified heme oxygenase–1 (HO-1), a stress-responsive enzyme displaying immunoregulatory and cytoprotective properties, as the main mechanism by which MDSC regulated alloreactive T cells. Although HO-1 has not been linked to MDSC before, its expression in allografts was associated with improved graft survival, and transfer of the HO-1 gene facilitated tolerance induction. HO-1 activity prevented DC maturation and proinflammatory cytokine production while preserving IL-10 secretion. Tin protophophyrin, a specific inhibitor of HO-1, prevented T-cell suppression and IL-10 production, whereas neither iNOS nor Arg-1 inhibition had any effect. HO-1 inhibition initiated before MSDC transfer prevented prolonged graft survival, suggesting that this pathway was important for the induction of transplantation tolerance [61].

Another recent report demonstrated that repeated injections of LPS combined with IFN-γ resulted in the expansion of two distinct cell populations in normal mouse spleen but not in lymph nodes. Both $Gr\text{-}1^{low}$ $CD11b^{high}$ $Ly6C^{high}$ SSC^{low} monocyte-like and $Gr\text{-}1^{high}$ $CD11b^{low}$ granulocyte-like MDSC populations inhibited DC development, release of NO

and CD8 proliferation. These data indicate that the spleen of healthy mice contains at least two subsets of MDSC, and combined LPS and IFN-γ treatment expand and activate MDSC [62].

In a skin graft model, engagement of immunoglobulin-like transcript (ILT)–2 receptor, an inhibitory receptor whose activation results in decreased T-cell activation, expanded a population of MDSC with enhanced suppressive activity. The most pronounced effect was found after binding of human leukocyte antigen–G to its receptor in ILT-2 transgenic mice. Skin allograft survival was prolonged after adoptive transfer of MDSC, and histologic evaluation of the allografts showed that the MDSC from ILT-2 transgenic mice migrated to the graft. Expansion of MDSC depended on Lyn kinase, tyrosine phosphatase, and Csk kinase activation; and the HLA-GILT-2 interaction induced the VEGF and GM-CSF that contributed to MDSC expansion. ILT-2 transgenic mice had increased expression of Arg-1, most likely resulting from upregulated IL-4 and IL-13 in MDSC [63].

Recently, we have also identified that monocytic MDSC play an essential role in an allogeneic cardiac transplantation model, using CD40 ligand and donor-specific splenocyte transfusion (DST) to induce tolerance in the recipient [79]. Monocytic MDSC may be essential for the induction of immune suppression in organ transplantation or graft-versus-host responses. Granulocytic MDSC, by contrast, may induce nonspecific immune suppression, which may suppress the effector phase of the allogeneic immune response at an early stage. It does, however, appear that to establish long-term antigen-specific tolerance and Treg development, the monocytic MDSC may be the key subset [64–66].

6. *In vitro* generation of MDSC

At present, methods for therapeutic targeting of MDSC are primarily focused on inhibiting the suppressor activity of these cells in tumor bearing hosts. This can be achieved by interfering with different aspects of MDSC biology. Drugs can be used to inhibit MDSC development from precursors or to selectively induce myeloid cell apoptosis (Table 3) [49,67–69]. Similarly, MDSC accumulation in peripheral organs can be prevented by blockade of tumor factors [17,70]. Inhibition of intracellular effector pathways by ROS inhibitors is another promising approach to block MDSC-mediated suppression of the antitumor immune response [71–73].

In transplantation it may be necessary to induce, expand, and activate these cells. One approach is adoptive transfer of *in vitro*–generated MDSC to control alloantigen-specific responses or to induce tolerance. Recently, our study demonstrated that functional MDSC could be successfully generated from both murine embryonic stem cells and bone marrow hematopoietic stem cells. Phenotypic analysis revealed two subpopulations: $CD115^+Ly^-6C^+$ and $CD115^+Ly^-6C^-$ cells. The first set corresponded to the monocytic $Gr\text{-}1^+CD115^+F4/80^+$ MDSC subset found in tumor-bearing mice, whereas the $CD115^+Ly^-6C^-$ cells were similar to granulocytic and monocytic progenitors. Functionally, the cells strongly inhibited T-cell proliferation induced by polyclonal stimuli or alloantigen *in vitro* and induced the development of $CD4^+CD25^+Foxp3^+$ regulatory T cells. Effector mechanisms included iNOS-mediated NO production and IL-10 release. Adoptive transfer of embryonic stem cell-derived MDSC successfully prevented graft-versus-host disease in more than 90% of recipients. These results offer a possible source for *in vitro* generation and use of MDSC [78]. Techniques to generate MDSC *in vivo* without engaging strong inflammatory stimuli remain to be developed.

7. Summary and perspective

MDSC represent a set of natural regulatory cells with a strong ability to control the adaptive immune response. Their potential role in the induction and maintenance of experimental and clinical transplantation tolerance requires far more investigation. For example, some transplant recipients become tolerant to their allografts during episodes of overwhelming sepsis while immunosuppression is withdrawn. MDSC may be generated during these inflammatory conditions, and may provide a major contribution to tolerance. The lineage and phenotype of human MDSC subsets must be defined in more detail, and the factors involved in the induction, expansion, and activation of MSDC during transplantation have not yet been explored. The migratory features and kinetic changes in the distribution and activity of MSDC, particularly in the context of transplantation, are not known.

In the chronic setting, such as long-term allograft survival, crosstalk between innate and adaptive immunomodulatory mechanisms might be required to ensure integrated control of immune reactivity and prevent allograft damage. Conversely, proper control of MDSC development or elimination is essential in maintaining the host antitumor immune response. Although current results implicate CTLA4-B7–based interactions as a possible molecular bridge between these arms of immunity, a better understanding of the interplay between Treg and innate suppressors is critical. For human applications, treatment regimens will have to be developed that control MDSC *in vitro* or *in vivo* by manipulating recipient myelomonocytic precursor cells.

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Table 1

Phenotype of MDSC

	Mouse	Human
Membrane markers (common)	Gr-1, CD11b	CD33, CD11b, CD34, low MHC class II
Granulocyte-like subset	$CD11b^{+}Ly6G^{+}Ly6C^{low}$ $CD11b^{+}$ $Gr\text{-}1^{high}$ $CD49d^{-}$	$CD33^{+}$, $CD15^{+}$, HLA-DR low
Monocyte-like subset	$CD11b^{+}Ly6G^{-}Ly6C^{high}$ $CD11b^{+}Gr\text{-}1^{int}$ $CD49d^{+}$	$CD33^{+}CD14^{+}$ HLA-Dr low

Table 2

Suppressor functions of MDSC

	Pathway	Effector molecule	Function
Granulocyte-like subset	STAT3, NADPH	High ROS, Low NO, Arg-1	Modification of T-cell receptor, antigen-specific unresponsiveness, nonspecific immune suppression
Monocyte-like subset	M1: STAT1, iNOS	M1: High NO, TNFα, Low ROS	Inhibition of MHC class Il expression, T-cell apoptosis
	M2: STAT 3, Arg-1	M2: High Arg-1, IL-10, TGFβ	Activation of Treg, induction of T-cell anergy, long-term tolerance

Table 3

Reagents and factors involved in the modulation of MDSC-mediated immune suppression or the elimination of MDSC

Effector	Suppresses allograft rejection	Enhances immune response	Reference
Anti-CD40 ligand	Yes		[74,75]
Anti-CD28	Yes		[59]
Glatiramer	Yes		[76]
JAK3 inhibitor	Yes		[77]
HLA-G	Yes		[63,74]
Progenipoietin-1 (synthetic G-CSF/Flt-3 ligand)	Yes		[64]
Sunitinib		Yes	[37,78,79]
Anti-MCSF		Yes	[70]
STAT3 inhibitor		Yes	[80]
5-Fluorouracil		Yes	[69]
Gemcitabine		Yes	[68]
Tumor-derived exosomes		Yes	[67]
Sildenafil		Yes	[49]