Reviews

The Role of Myeloid-Derived Suppressor Cells in Viral Infection

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Abstract

Myeloid-derived suppressor cells (MDSCs) are heterogeneous immature myeloid cells that are well described as potent immune regulatory cells during human cancer and murine tumor models. Reports of MDSCs during viral infections remain limited, and their association with immunomodulation of viral diseases is still being defined. Here, we provide an overview of MDSCs or MDSC-like cells identified during viral infections, including murine viral models and human viral diseases. Understanding the similarities and/or differences of virally induced versus tumor-derived MDSCs will be important for designing future immunotherapeutic approaches.

Keywords: MDSCs, viral infections, immunotherapeutic approaches

Introduction

Myeloid-derived suppressor cell phenotype

THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs) during viral infection is not well defined; however, MDSCs have been heavily studied in the context of human cancer and murine tumor models and have been revealed to contribute to the immunosuppressive tumor microenvironment [reviewed in Refs. (10,101,150)]. MDSCs dampen T cell-mediated immune responses against several types of cancer, including melanoma, and lung, mammary, and colon carcinomas (94,140,143). These myeloid cells, or a subset, may have been initially included in studies that identified natural suppressor (NS) cells capable of inhibiting cytotoxic T lymphocyte (CTL) activity. MDSCs are currently well characterized as primary cellular immune modulators of the tumor microenvironment, along with CD4+ regulatory T cells (Tregs) (4,101,150,).

The nomenclature used to categorize these heterogeneous cell populations has been diverse, including NS cells, immature myeloid cells (IMCs), and myeloid suppressor cells, until reaching a consensus of ''myeloid-derived suppressor cells'' (46,150). Murine MDSCs are phenotypically defined as CD11b+GR1+. Because the anti-GR1 mAb binds both Ly6C and Ly6G, MDSCs are further subdivided into Ly6G−/lowLy6C+/high monocytic (M-MDSCs), which appear similar to monocytes with a large, round nucleus, and Ly6G+/high-Ly6C−/low granulocytic (G-MDSCs or PMN-MDSCs), which have been described as having a multilobed nuclei somewhat similar to those seen in polymorphonuclear cells (26,47,115,150).

A recent review, primarily focused on MDSCs in tumor systems, has advocated the use of the term PMN-MDSC over G-MDSC (10). However, most current publications refer to these cells as G-MDSCs. A third subset, termed immature or early-stage MDSCs, has been observed by several groups in human cancers, but a murine equivalent has not yet been identified, and there are few if any reports of this subset in viral infections (10,93). Murine MDSCs display varying coexpression of other surface markers, including but not limited to, TLR4, F4/80, FcγRIII/II (CD16/32), IL-4Rα (CD124), and/or CD115 (47,94,140).

Phenotypic identification and classification of human MDSCs are more difficult, but the general consensus definition is CD33+CD11b+LIN−HLA-DR− cells. Human MDSCs can be further subdivided into CD14+/dull M-MDSCs and CD14−CD15+ G-MDSCs (150), although variations in these definitions remain. Additional information about the different phenotypes attributed to human MDSCs can be found in the 2012 review by Poschke and Keissling (121). Murine and human MDSCs may also express different combinations of chemokine receptors needed for egress of MDSCs out of the bone marrow and/or migration to tumor sites or sites of infection, such as CCR2, CXCR4, CXCR2, and/or CX3CR1 (9,58,62,68,86,111,112,133,138,173,176).

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As IMCs, MDSCs share phenotypic characteristics with other innate immune cells, including neutrophils, dendritic cells, macrophages, or monocytes, leading to variations in MDSC nomenclature. Although there may well be some plasticity associated with a continuum of many of these cells, murine MDSCs have increased surface expression of GR1 and are functionally immature compared to tumor-associated macrophages (TAMs) (123,150).

As the cell surface phenotype of MDSCs is similar to that of other cells (i.e., monocytes and neutrophils), it is critical to show suppressive function to properly identify MDSCs, at least until unequivocal MDSC markers are identified (10). Because quantifying *in situ/in vivo* suppressive function is generally challenging, MDSCs are typically isolated *ex vivo* from virus-infected hosts or derived *in vitro* from precursor cells, and suppression is measured by their inhibition of cytokine production and/or proliferation of responder T cells (10). Cells that meet the phenotypic criteria and are suppressive of (at least) T cell functions can be documented as MDSCs (10).

Alternatively, if suppressive activity is not determined, but biochemical characteristics indicate the cells may be MDSCs (including transcription factor and regulator expression (e.g., IRF8, phospho-STAT3, c/EBPβ, S100A8/A9, or Rb1), upregulation of cytokines and their receptors, and/or presence/production of immune-regulatory molecules), cells should be recognized as MDSC-like cells (MDSC-LCs) (10). A detailed description of the criteria for identifying MDSCs can be found in the referenced review (10). In this context, MDSCs during viral infection may be substantially underreported and/or identified as other myeloid cell types. Furthermore, MDSC-LCs have been phenotypically identified in several viral systems, but often were not tested for suppressive activity. The following discussion identifies studies that demonstrate or support a role of MDSCs or MDSC-LCs (as defined by phenotype and/or function) during viral infection.

Immune Target Cell Specificity of MDSCs

MDSCs or MDSC-LCs isolated from virus-infected patients and/or from animal viral models suppress several immune cell targets and use a variety of immunosuppressive mechanism(s) to modulate immune responses (Table 1).

T cells

MDSCs from a variety of viral infections, including but not limited to nonretroviral RNA viruses as featured below, are able to suppress T cell responses. MDSCs isolated from hepatitis C virus (HCV)-infected patient peripheral blood mononuclear cells (PBMCs) or derived *in vitro*, by coculturing CD33$^+$ mononuclear cells with hepatocytes infected with HCV or treated with HCV core protein, inhibited polyclonal CD4$^+$ and CD8$^+$ T cell proliferation and production of interferon (IFN)-γ and IL-2 (14,148). Lung MDSCs from influenza A virus (IAV)-infected mice and CD11b$^+$ cells isolated from the PBMCs of patients with a recent IAV infection inhibited Ag-specific proliferation of T cells (31).

Interestingly, splenic M-MDSCs from mice infected with the C13 chronic lymphocytic choriomeningitis virus (LCMV) strain, but not the Armstrong acute LCMV strain, suppressed Ag-specific CD8$^+$ T cell proliferation (109). These data may

indicate that chronic inflammation is a driver for MDSC development. Alternatively, expansion of MDSCs driven by immune responses to some, but not all, acute infections may contribute to the development of chronic inflammation.

Retroviral infection also induces MDSCs. *In vitro* derived MDSCs, via stimulation of PBMCs with the HIV glycoprotein 120 (gp120), or *ex vivo* MDSCs derived from HIV-infected patient blood inhibited polyclonal and Ag-specific CD4$^+$ and CD8$^+$ T cell proliferation and IFN-γ production (8,50,122,155) and increased FoxP3$^+$ CD4$^+$ Treg differentiation (159). In these studies, inhibitory activity was independent of the phenotype of the MDSCs, which were either monocytic like (8,50,122) or granulocytic like (155).

Similarly, M-MDSCs isolated from circulation in simian immunodeficiency virus (SIV)-infected macaques inhibited polyclonal and Ag-specific CD8$^+$ T cell proliferation (146). During infection with LP-BM5, a murine retrovirus, which causes profound immunodeficiency similar to HIV/AIDS (termed MAIDS) (2,15,153), and splenic M-MDSCs inhibited polyclonal CD4$^+$ and CD8$^+$ T cell proliferation and IFN-γ production (53).

MDSCs have also been indentified during DNA virus infection. MDSCs accumulated in hepatitis B virus (HBV)-infected patients, with preferential expansion of G-MDSCs, rather than M-MDSCs, both in circulation and in the liver (92,116). *Ex vivo* G-MDSCs from HBV-infected patients or derived from a murine model of HBV infection, inhibited Ag-specific and polyclonal CD4$^+$ and CD8$^+$ T cell proliferation and IFN-γ production (20,116).

Inflammatory monocytes, with an M-MDSC phenotype, derived from murine cytomegalovirus (MCMV)-infected mice also suppressed *ex vivo* MCMV-specific CTL responses and cytokine polyfunctionality (30). MDSCs or MDSC-LCs accumulated in HSV-1-infected corneas (34,130,154), but it remains unclear whether these cells demonstrated inhibitory activity. In one study, F4/80$^-$GR1$^+$ cells accrued in the corneas of HSV-1-infected mice and the authors concluded, based on nuclear morphology, that these infiltrating cells were not MDSCs, however, functional studies were not provided (130).

CD4$^+$ T cell functional and phenotypic subsets

In addition to suppressing CD4$^+$ and CD8$^+$ T cell proliferation and IFN-γ production, MDSCs can also modulate CD4$^+$ T helper (Th) cell polarization. IL-10 and TNF-α produced by MDSCs isolated from IAV-infected mice mediated skewing to CD4$^+$ Th2 cells (72).

Alternatively, incubation of PBMCs with MDSCs derived from HCV-infected patients resulted in increased numbers of Tregs (128). Increased Treg frequencies have been observed in HIV-infected patients and were correlated with increased MDSC frequency (155). Furthermore, M-MDSCs derived *in vitro* or isolated from HIV-infected patients induced CD4$^+$ FoxP3$^+$ Treg expansion and production of IL-10 by CD4$^+$ T cells (presumably by these Tregs) *in vitro* (50,155). In the mouse retrovirus immunodeficiency system, *ex vivo* derived M-MDSCs from LP-BM5-infected mice also induced a moderate expansion of Tregs *in vitro*, but concurrently suppressed their IL-10 production (114). Together, these studies support a relationship between CD4$^+$ FoxP3$^+$ Tregs and MDSCs in viral systems.

TABLE 1. OVERVIEW OF MDSCs AND MDSC-LCs IDENTIFIED IN VIRUS INFECTIONS

Virus		Host	Location	Phenotype	Identified as MDSC by authors	Cellular targets of suppression	Suppressive mechanisms	Refs
RNA viruses								
(−)ssRNA	IAV	Mouse	Lung	CD11b$^+$ GR1$^+$	Yes	CD8$^+$ T cells	iNOS products, Arg1	(31)
(−)ssRNA	LCMV	Mouse	Spleen	GR1^{int} Ly6C^{hi}	Yes, monocytic	CD8$^+$ T cells	iNOS products	(109)
(+)ssRNA	HCV	Human	PBMC	CD11b$^+$ CD33$^+$ HLA-DR$^{−/low}$ CD14$^+$	Yes, monocytic-like	CD4$^+$ and CD8$^+$ T cells	Arg1, H$_2$O$_2$	(14,52,106,148,170)
				CD66b$^+$	Yes, granulocytic	CD8$^+$ T cells	ND	(107)
(+)ssRNA	TMEV	Mouse	CNS	CD11b$^+$ GR1$^+$; CD11b$^+$ GR1$^−$ Ly6G$^−$	Yes	ND	ND	(7)
Retroviruses								
(+)ssRNA	HIV	Human	In vitro	CD11b$^+$ CD33$^{+/high}$ HLA-DR$^{−/lo}$ CD14$^+$ CD15$^−$	Yes, monocytic-like	CD4$^+$ and CD8$^+$ T cells	iNOS products, H$_2$O$_2$, Arg1	(50,122)
			PBMC	CD11b$^+$ CD33$^{+/high}$ HLA-DR$^{−/lo}$ CD14$^+$ CD15$^−$; CD14^{high} CD16$^−$ CCR2$^{−/low}$	Yes, monocytic-like	T cells	Arg1	(49,122)
				CD11b$^+$ CD33$^+$ CD14$^−$ CD15$^+$; CD11b$^+$ CD15$^+$ CD33$^+$ CD66b$^+$	Yes, granulocytic	T cells	ROS, PD-L1/PD-1	(8,24,155)
(+)ssRNA	SIV	Macaque	PBMC	CD11b$^+$ CD33$^+$; CD14^{high} CD16$^−$ CCR2L$^{−/low}$	Yes	CD8$^+$ T cells	May be iNOS products (iNOS is expressed)	(49,146)
(+)ssRNA	LP-BM5 Retrovirus	Mouse	Spleen	CD11b$^+$ Ly6C$^+$ Ly6G $^{±/low}$	Yes, monocytic	T cells, B cells	VISTA, NO, SO, PNT, TGF-β	(53,54,112,114,124)
DNA viruses								
DNA	HBV	Mouse	Liver	CD11b$^+$ GR1^{high}	Yes	T cells	ND	(20,76)
		Human	PBMC; Liver	CD11b$^+$ CD33$^+$ HLA-DR$^−$; CD11b$^+$ CD33$^+$ HLA-DR$^−$ CD14$^−$ CD15$^+$	Yes, granulocytic	CD4$^+$ and CD8$^+$ T cells	Arg1, PD-L1, IL-10	(39,66,92,116)
DNA	VV	Mouse	Spleen	CD11b$^+$ Ly6C^{low} Ly6G$^+$	Yes, granulocytic	NK cells	H$_2$O$_2$	(42)
DNA	Recombinant adenovirus	Mouse	Spleen	CD11b$^+$ Ly6C^{lo} Ly6G$^+$	Yes, granulocytic	NK cells	H$_2$O$_2$	(174)
DNA	CMV	Mouse	Spleen	CD11b$^+$ Gr1^{lo} Ly6C^{hi}	No, inflammatory monocytes	CD8$^+$ T cells	iNOS products (including NO)	(30)
DNA	HSV-1	Mouse	Cornea	CD11b$^+$ GR1$^+$	Yes	ND	ND	(131)
DNA	HSV-2	Mouse	Skin	CD11b$^+$ Ly6G$^+$; GR1^{bright}	No, neutrophils	ND	ND	(34,154)
				GR1$^+$	No, neutrophils	ND	ND	(40)
DNA	MHV-68	Mouse	Spleen	CD11b$^+$ GR1$^+$	MDSC-like	ND	ND	(104)

Arg1, arginase 1; CMV, cytomegalovirus; CNS, central nervous system; H$_2$O$_2$, hydrogen peroxide; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; IAV, influenza A virus; iNOS, inducible nitric oxide synthase; LCMV, lymphocytic choriomeningitis virus; LP-BM5, murine γ retrovirus; MDSC-LC, myeloid-derived suppressor cell-like cells; MHV-68, murine gammaherpesvirus 68; ND, not determined; NK cells, natural killer cells; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; PNT, peroxynitrite; ROS, reactive oxygen species; SIV, simian immunodeficiency virus; SO, superoxide; TGF-β, transforming growth factor beta; TMEV, Theiler's murine encephalomyelitis virus; VISTA, V-domain Ig suppressor of T cell activation; VV, vaccinia virus.

B cells

Despite the large number of studies in tumor systems that demonstrate MDSC-mediated suppression of T cell responses, only a few reports have discussed the ability of myeloid-lineage cells to suppress B cell responses: for example, in two early studies by Mac-1$^+$2$^+$ MDSC-LCs (44,162). More recent study showed that MDSCs in the bone marrow of aged mice suppressed B cell lymphopoiesis in an IL-1-dependent manner (74,75). B cells play crucial roles in cancer as sources of malignant cells in several lymphomas (136) and as sources of tumor-specific antibodies (144). In autoimmunity, B cell self-tolerance is frequently defective, causing production of pathogenic, autoreactive antibodies and increases in the size of the memory B cell pool (35). M-MDSCs in a collagen-induced arthritis model were shown to suppress both T and B cells (29).

More relevant to this review, in viral disease, B-lineage cells play a variety of crucial roles: plasma cells produce antibodies to prevent or contain viral infection, and memory B cells allow for fast, more adaptive antibody production following rechallenge (36). In the mouse retrovirus immunodeficiency system, MAIDS, M-MDSCs derived from LP-BM5 retrovirus-infected mice suppressed polyclonal B cell proliferative and IL-10 producing B-regulatory (Breg) responses (53,124). Of note, no suppression of B (or T) cell responses was observed by Ly6G-enriched splenocytes (which contain G-MDSCs) in this system (53). To our knowledge, suppression of the B cell compartment has only been observed by M-MDSCs, and studies have not yet identified G-MDSC suppression of B cells.

Other cellular targets

G-MDSCs from vaccinia (42) and adenoviral vector (174)-infected mice inhibited natural killer (NK) cell responses. Whether MDSCs from these two models also suppress T or B cell responses remains to be determined. In a mouse mammary tumor model, MDSCs impaired antitumor T cell responses by direct suppression of T cells and also by indirect modulation of macrophages that skewed the T cell milieu (141). However, to our knowledge, a similar interaction between MDSCs and macrophages has not been described during viral infections.

Early Studies of Molecular Mechanisms of MDSC Suppression: T Cell Targets

The major mechanisms of suppression by MDSCs, primarily discovered in tumor systems, include catabolism of L-arginine (L-Arg) by arginase 1 (Arg1) and/or inducible nitric oxide synthase (iNOS); production of reactive oxygen species (ROS); production of immunosuppressive cytokines; or negative checkpoint regulators (i.e., V-domain Ig suppressor of T cell activation [VISTA], also known as PD-1H) (41,54,158).

Broadly, G-MDSCs and M-MDSCs preferentially express Arg1 or iNOS, respectively, but both enzymes can be coexpressed within the same cell (26). Several cross-inhibitory interactions have been reported between the Arg1 and iNOS pathways (12,13,125). Arg1 has a lower affinity for L-Arg, but catalyzes the reaction faster than iNOS, and thus, their overall rates of conversion of L-Arg are relatively similar (12). Understanding the requirements of expression of Arg1 and/or iNOS, including gene expression, cytokine inducibility, and/or metabolic programming, is needed to determine the fine regulation of these two suppressive mechanisms utilized by MDSCs.

Inducible nitric oxide synthase

There are three isoforms of NOS: NOS1 or neuronal NOS (nNOS), NOS2 or iNOS found in myeloid cells, and NOS3 (eNOS) found in endothelial cells (12,163). iNOS expression in myeloid cells is driven by Th1 cytokines, including IFN-γ, as well as by IL-1, TNF-α, IFN-α, and IFN-β (12). Metabolism of L-Arg by iNOS produces NO and citrulline (125,129). NO-mediated suppression may be caused by direct S-nitrosylation of cysteine residues and/or indirect activation of soluble guanylate cyclase and cyclic-GMP-dependent protein kinases, which alter IL-2 signaling in T cells (12).

Arg1

Of two isoforms, Arg2 is constitutively expressed in mitochondria of a variety of nonhepatic cells, including renal cells, neurons, macrophages, and enterocytes, with limited expression in the liver (12,163). In contrast, Arg1, located in the cytoplasm of hepatocytes and myeloid cells, including MDSCs (12,163), is cytokine inducible (by Th2 cytokines, including IL-4, IL-13, and IL-10) in mouse myeloid cells, but is constitutively expressed in human myeloid cells (12,163). It is tempting to speculate that the constitutive expression of Arg1 in human MDSCs may predispose them to favor suppression by Arg1, as opposed to iNOS, however, this has not been definitively shown. The main mechanism by which arginine catabolism inhibits T cell function may be by depletion of extracellular L-Arg and urea production, resulting in loss of CD3ζ expression, but other mechanisms, such as decreased mRNA translation, have been considered (12).

Reactive nitrogen and oxygen species

Low L-Arg concentrations can also promote production of superoxide anion (O$_2^-$), which may act on its own but can further participate in the formation of reactive nitrogen species (RNS) and other ROS (12). Cytokines such as transforming growth factor beta (TGF-β), IL-3, IL-6, and IL-10 induce ROS production by MDSCs (132). O$_2^-$ can interact with NO to form peroxynitrites (ONOO$^-$) or with protons to form hydrogen peroxide (H$_2$O$_2$) (12). RNS and ROS promote apoptosis of target cells by inducing post-translational protein modifications, including nitrating tyrosine residues, or increasing CD95 and downregulating BCL-2 in activated cells (12,163). High ROS production may prevent differentiation of IMCs, leading to increased MDSC numbers as a positive feedback loop (79).

Other mechanisms

Other mechanisms of suppression associated with MDSCs in tumor or other nonviral systems include upregulation of negative checkpoint regulators, such as PD-1/PD-L1, on MDSCs from cancer patients (3,171). MDSC-mediated suppression was shown to be PD-L1 dependent in a study of immunotherapy for human colorectal cancer liver metastasis (152) as well as in a study of *pneumocystis* pneumonia (85).

MDSCs can also suppress cells by the production of immunosuppressive cytokines: for example, MDSC-derived IL-10 was shown to suppress T cell and macrophage effector

function in murine models of ovarian cancer, mammary carcinoma, and colorectal carcinoma (5,57). Furthermore, in the ovarian cancer model, IL-10 signaling to MDSCs through IL-10R was required for the suppressive phenotype of the MDSCs (57). MDSC IL-10 production was also implicated in the resolution of inflammation in bacterial pneumonia (120). Another immunosuppressive cytokine, TGF-β, was produced by MDSCs in several murine tumor models (23,87,88,168).

MDSC Suppression in Viral Infections: Target Cells and Mechanisms

Nonretroviral RNA viruses

The mechanisms mentioned above are used either individually or in combination by MDSCs during infection with viruses, including nonretroviral RNA viruses discussed below (Table 1). In HCV, MDSC frequencies from chronically infected patients directly correlated with liver Arg1 levels (170). MDSCs from HCV-infected patients inhibited *ex vivo* CD4$^+$ and CD8$^+$ T cell responses via Arg1 (14). In another study, MDSCs, induced by exposing PBMCs to HCV, suppressed NK cells, also via Arg1 (52). Decreased TCRζ expression was observed on liver CD8$^+$ T cells from chronically infected HCV patients, whereas expression increased in infected patients receiving pegylated-IFN-α/ribavirin antiviral therapy (170). TCRζ expression was restored in *ex vivo* T cell cultures from HCV-infected patients when supplemented with L-Arg (170).

Another group observed increased ROS production by cells with an M-MDSC-like phenotype in the peripheral blood of chronically infected HCV patients, but did not confirm their *ex vivo* suppressive capability (148). In the same study, M-MDSCs derived *in vitro*, by coculturing CD33$^+$ mononuclear cells with HCV core protein or hepatocytes infected with HCV, utilized ROS to suppress T cells (148).

CD11b$^+$ cells isolated from the peripheral blood of patients with a recent IAV infection suppressed T cells in an iNOS- and Arg1-dependent manner, which was also observed for MDSCs from IAV-infected mice (31). Inhibition of immune responses by M-MDSCs from LCMV C13 chronically infected mice was dependent on iNOS (109).

Retroviruses

In the handful of recent studies of MDSCs derived *ex vivo* from HIV-infected patients (8,122,155) or induced *in vitro* by stimulation of PBMCs with HIV gp120 (50), the mechanisms of immune suppression appear to be diverse, including iNOS (50), ROS (50), Arg1 (122), and the PD-L1/PD-1 pathway (8), while in some cases the mechanism(s) involved were not examined (155). In most cases, these cells displayed a monocytic-like phenotype (8,50,122), however, a granulocytic-like phenotype was observed in one study (155). The mechanism of suppression of T cell responses by M-MDSCs from SIV-infected macaques has not been definitively determined, but may be linked to high iNOS expression by the MDSCs (49,146).

M-MDSCs derived from mice infected with LP-BM5 retrovirus suppressed T cell responses in a predominantly iNOS/NO-mediated manner (53). Genetic ablation of indoleamine 2,3-deoxygenase (*IDO*) did not affect MDSC-mediated suppression; however, *IDO* may function in wild-

type MDSCs from LP-BM5-infected mice and be compensated for by other mechanisms in the *IDO*-knockout MDSCs (113). Supporting a potential role for *IDO* in LP-BM5, overall *IDO* is increased during MAIDS and may play a role in the associated pain hypersensitivity (65,69,113). Current data are conflicting as to whether increased *IDO* plays a role in LP-BM5 retrovirus replication and immunodeficiency (65,113).

Suppression of B cell responses by these same M-MDSCs from LP-BM5-infected mice was mediated not only, in part, by iNOS/NO (53) but also substantially by the negative checkpoint regulator VISTA (41,54,158), and by ROS, RNS, and TGF-β (Fig. 1a) (124). Further division of M-MDSCs from LP-BM5-infected mice into subpopulations revealed differential dependence on iNOS-mediated suppression (112) and highlighted the phenotypic and functional heterogeneity of these cells (Fig. 1b).

A pie chart showing the different mechanisms used by M-MDSCs from LP-BM5 infected mice and their relative contributions to suppression of B cells can be seen in Figure 2. The studies of M-MDSCs in the LP-BM5 system highlight several more general characteristics of MDSCs, including having multiple cellular targets, contact-dependent and soluble suppressive mechanisms, and heterogeneity of phenotype and mechanism among subsets.

DNA viruses

MDSCs from patients and mice infected by DNA viruses have also been shown to be capable of expressing Arg1 and iNOS, and to produce ROS. In HBV-infected patients, G-MDSC frequency correlated positively with increased Arg1 concentrations and inversely with CD3ζ expression on T cells (116). Consistent with this observation, *ex vivo* G-MDSCs from HBV-infected patients in the same study suppressed *in vitro* T cell responses in an Arg1-dependent manner (116). Another study, however, found that MDSCs from chronically HBV-infected patients suppressed CD8$^+$ T cell proliferation, IFN-γ production, production of granzyme B and perforin, and degranulation in a manner dependent on PD-1-induced IL-10 production (66). In the HBV transgenic murine model of persistent HBV infection without significant pathology, in which the complete HBV genome is injected into fertilized eggs, *ex vivo* Arg1- and iNOS-dependent suppression of T cells by MDSCs was observed (20,76).

G-MDSCs from vaccinia- or adenovirus-infected mice used ROS to suppress NK cells (42,174), but it remains to be determined whether ROS production by these cells also modulated other immune cell responses.

Despite early identification of cells with an MDSC-like surface phenotype in murine corneal HSV-1 infection, suppressive activity of these cells, to our knowledge, was not reported (131). In another study, total corneal iNOS and Arg1 mRNA levels increased during murine HSV-1 infection (98). Increased NO and H_2O_2 production by human PMNs (with MDSC-like properties) was observed when incubated with the supernatants of corneal cells treated with HSV components (60). Collectively, these data suggest that MDSC-LCs derived during HSV infection may be able to utilize several mechanisms to suppress inflammation.
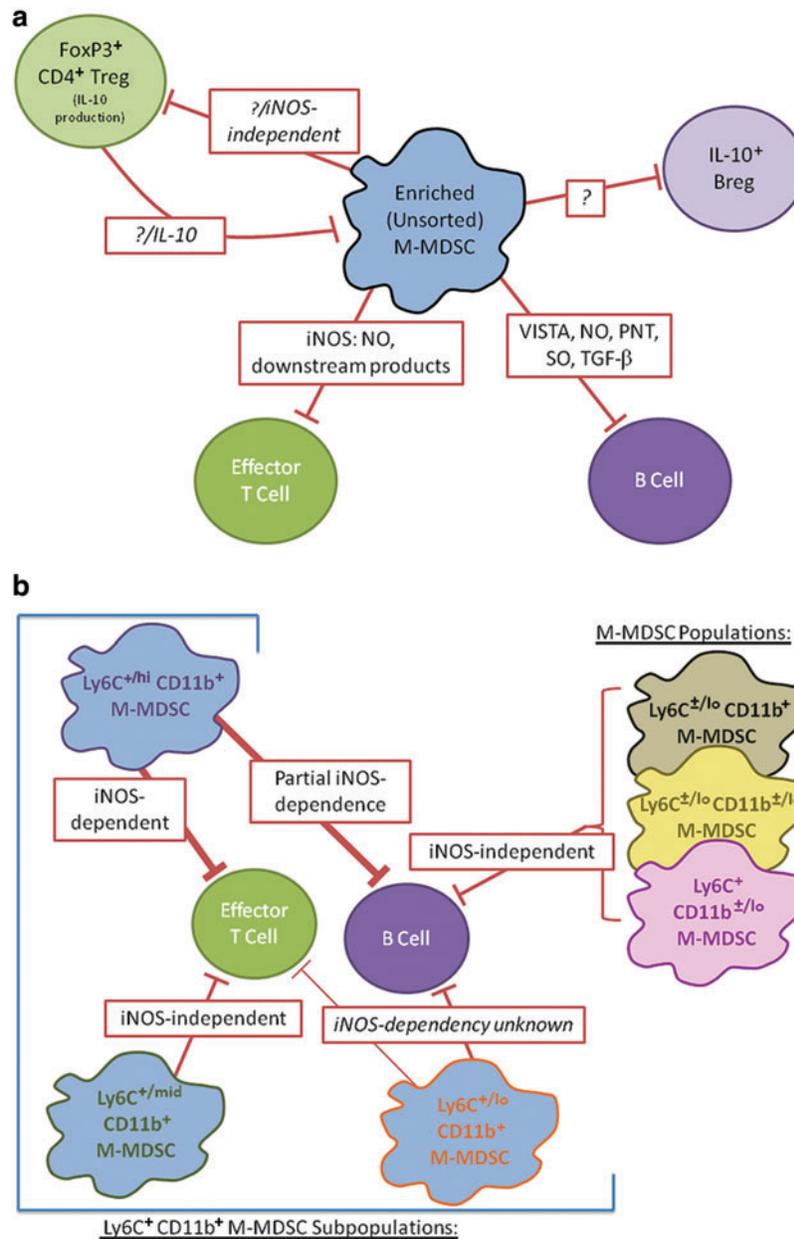
FIG. 1. Overview of interactions between M-MDSCs, Tregs, and immune cell targets during LP-BM5 infection. **(a)** *Lines* indicate suppressive relationships based on *in vitro* (M-MDSC-mediated suppression) and *in vivo* (CD4$^+$ Treg) studies. Mechanisms are listed in *red boxes*, *question marks* indicate unknown mechanisms (53,54,114,124). **(b)** M-MDSCs were sorted based on their cell surface expression of Ly6C and CD11b (112). *Lines* indicate suppressive relationships based on *in vitro* studies, with line thickness reflecting the magnitude of the suppression. The iNOS dependency of these relationships is listed in *red boxes*, with unknown dependence in *italics* (112). iNOS, inducible nitric oxide synthase; MDSCs, myeloid-derived suppressor cells; PNT, peroxynitrite; SO, superoxide; TGF-β, transforming growth factor beta; Tregs, regulatory T cells; VISTA, V-domain Ig suppressor of T cell activation. Color images available online at www.liebertpub.com/vim

In the murine gammaherpesvirus (MHV-68) model, splenic CD11b$^+$GR1$^+$ cells accumulated during the latent stage of infection, with a preferential accumulation of Ly6G$^+$ cells (104). However, these cells from MHV-68-infected mice did not demonstrate suppressive activity *ex vivo* (104), implying they were not MDSCs and/or may not modulate antiviral activity in this system. Furthermore, cultures containing CD11b$^+$GR1$^+$ cells from MHV-68-infected mice did not upregulate iNOS or Arg1 activity (104).

Relationships of MDSCs to Disease, and Intervention Strategies with MDSCs as Targets of Immunotherapy

MDSCs as correlates of disease

During viral infections, MDSC accumulation and/or activity often correlate with disease parameters. For example, increased MDSC frequency and Arg1 activity corresponded with the extent of HIV-related pathogenesis, including reduced CD4$^+$ T cell counts and increased viral load (24,25,122,155). MDSC
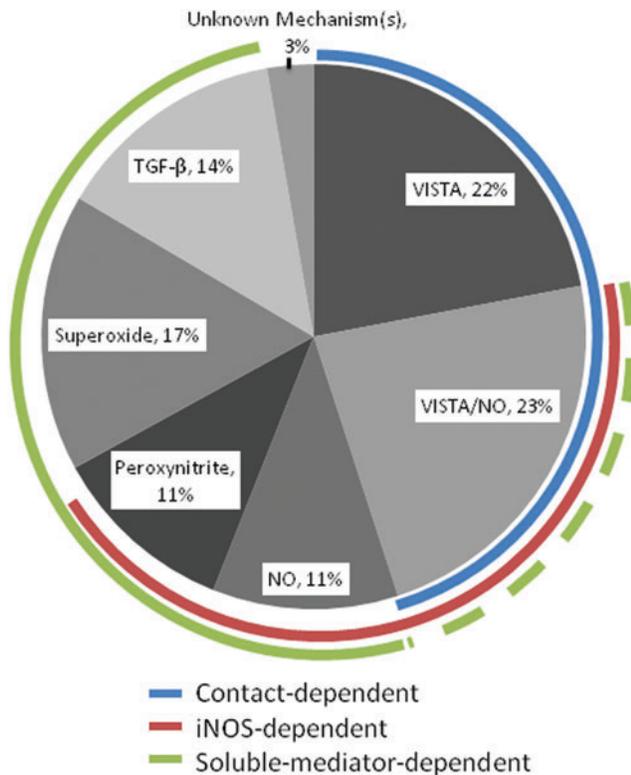
FIG. 2. M-MDSC mechanisms of B cell suppression. Based on the studies presented in references (53,54,124), including experiments showing additivity, known mechanisms of LP-BM5-expanded wild-type murine M-MDSCs are summarized with estimates of their relative contributions to their suppression of B cells (with acknowledgment of the difficulty in accurately summing up the proportionality of such mechanisms due to the likely plasticity and compensation of molecular suppressive mechanisms). Color images available online at www.liebertpub.com/vim

frequencies decreased on initiation of HAART in HIV patients and in SIV-infected macaques, further implicating MDSCs as indicators of HIV/SIV disease level (49,122,155). Similarly, antiviral therapy decreased MDSC frequencies in the blood of HCV patients (14). In the LP-BM5 MAIDS model, M-MDSC frequency also paralleled altered disease severity, with *PD-1*, *PD-L1*, and *IL-10* knockout mice showing increased disease severity along with increased MDSC frequency when compared with wild-type mice (53).

During HSV-1 and HSV-2 infection of mice, cells phenotypically resembling MDSCs infiltrated the cornea (34) and skin (40,164). In one study, increased herpes simplex keratitis (HSK) and viral titers were observed in HSV-1-infected Kit[W−sh] mice (that lack CD31+ mast cells), compared to wild-type mice (130). This increased disease correlated with increased influx of G-MDSC-like/PMN cells into the corneas of HSV-1-infected Kit[W−sh] mice (130), and implied that MDSCs may dampen the immune responses that normally reduce viral replication. However, functional assays are difficult to perform on MDSCs from the corneal and skin infection models due to their low frequency and challenges in isolation. It will be important to determine whether different subsets of MDSCs (or other myeloid cells) infiltrate to different sites of infection or in response to different HSV species.

Despite accumulation of MDSCs during viral infections, it remains unclear what role these cells play in these diseases. M-MDSCs accumulated within the liver (170) and PBMCs (14,52,106,128,149) of HCV-infected patients. Some studies found a correlation between M-MDSC levels and HCV viral load or other disease parameters [including two of the studies of the PBMC (14,52) and one of the liver (170)], while other studies of the PBMC compartment found no correlation (106,128).

MDSCs as targets of viral replication

In addition to suppressing antiviral immune responses, M-MDSCs can also be sites of viral replication. M-MDSCs from healthy and HIV-infected patients express the HIV-1 entry receptor CD4+, as well as the coreceptors CCR5 and CXCR4, rendering these MDSCs targets for HIV infection (122). M-MDSCs from healthy subjects could be infected *de novo* with HIV strains *in vitro*, and HIV *env* and *gag* genes were detected in MDSCs from HIV+ patients (122). In addition to being direct targets of HIV infection, MDSCs increased *ex vivo* viral replication in cocultured CD4+ T cells (122).

The ubiquitously expressed CAT-1 is the mouse cell receptor for LP-BM5 (59), and conceptually renders M-MDSCs, among many other cell types, susceptible to LP-BM5 infection. LP-BM5 retroviral gag gene expression (by qRT-PCR) and titers of LP-BM5 retrovirus infectious centers have readily been detected in highly enriched Ly6G-depleted, CD11b-enriched M-MDSCs from LP-BM5-infected mice (53). Collectively, these data support the idea that MDSCs may be active targets for retroviral replication.

Factors involved in MDSC expansion

Several tumor-derived factors may contribute to MDSC activation and recruitment, including prostaglandins, IL-6, IL-1β, IFN-γ stem cell factor, granulocyte macrophage colony-stimulating factor (CSF), macrophage CSF (M-CSF), vascular endothelial growth factor, and markers of cellular stress (i.e., MHC I chain-related molecule) (28,47,104,150,167). Prostaglandin E2 (PGE2) inhibited differentiation of dendritic cells (DCs) and instead caused IMCs to adopt an MDSC phenotype (110). A positive feedback loop between PGE2 and COX2 (a regulator of PGE2 synthesis) induced transcription of the immunosuppressive molecules *iNOS*, *IDO*, and *IL-10* (55,105, 151). IL-6 and M-CSF were also implicated in myeloid cell development and inhibited differentiation into DCs (43,45,77).

IL-6 levels have been shown to directly correlate with MDSC numbers *in vivo* (135). Many of these molecules signal through STAT3, which also upregulates S100A9, a myeloid-related protein that prevented DC maturation resulting in accumulation of MDSCs (21,103,166). IL-10, conversely, inhibited MDSC-expansion by blocking IL-6 signaling through STAT3 (83). Several of these and other proinflammatory and damage-associated signals are also upregulated during chronic viral infections, and may play analogous roles in MDSC expansion.

Changes to the tumor microenvironment, including acidosis, hypoxia, and alterations in glucose levels, can also modulate MDSC activity and phenotype (150). For example, hypoxia results in increased HIF-1α expression through decreased degradation and increased translation (169), which upregulated iNOS and Arg1 (27,81). Endoplasmic reticulum stress has also been shown to upregulate iNOS

and Arg1, as well as the ROS producing enzyme NADPH oxidase (82).

The factors involved in driving MDSC expansion and/or differentiation during viral infections, including cytokines and chemokines, remain less clear. During chronic HBV infection, the frequency of MDSCs correlated with the level of hepatitis B surface Ag (HBsAg), and culture of human PBMCs with HBsAg led to differentiation of these cells to MDSCs in an ERK/IL-6/STAT3 signaling-dependent manner, indicating that viral antigen may play a role in triggering the expansion of MDSCs (39).

Similarly, HCV core protein was able to induce generation of MDSCs from PBMCs by activating STAT3 and the PI3K pathway (118,148,149). During HCV infection, expression of several miRNAs was altered in $CD33^+$ myeloid cells, including miR-124, which was downregulated, in part, due to HCV-induced overexpression of STAT-3 (127). Downregulation of miR-124 correlated with increased levels of IL-10 and TGF-β expression by MDSCs (127).

In vitro, IL-6 mediated M-MDSC expansion from PBMCs by HIV gp120 stimulation (33,50). Direct HIV infection or the presence of the HIV-1-derived transcriptional transactivator (Tat) protein also induced M-MDSC expansion *in vitro* (122). Analysis of transcriptional gene targets of Tat pointed to IL-6 and TGF-β as candidates for mediating Tat-induced MDSC expansion (122). Immune activation of other cells by viruses may also cause MDSC expansion, as was seen with human hepatic stellate cells activated by inflammatory stimuli, which induced development of M-MDSCs through cell contact and CD44 (63).

In tumor systems, cross talk between Tregs and MDSCs was observed, and factors produced by Tregs, such as TGF-β or IL-10, modulated MDSC expansion, either directly or by changing the inflammatory milieu (68,89). In macaques vaccinated with a recombinant modified vaccinia Ankara (MVA) vector, increased plasma latent TGF-β was observed, which positively correlated with PBMC MDSC frequencies, compared to naive animals—all this consistent with a possible role for TGF-β in MDSC expansion (146). Additional unpublished data from the same group implied that neutralizing TGF-β, using an intrarectal anti-TGF-β Ab during MVA vaccination, reduced MDSC frequency. The source of the TGF-β, however, was not determined. Although $CD4^+$ Tregs are a potential source of TGF-β, no increase in $CD4^+$ Treg numbers was seen on vaccination (146). Nevertheless, the amount of TGF-β produced on a per Treg basis may have increased.

In the LP-BM5 murine retroviral system, M-MDSC frequency and suppressive activity by M-MDSCs increased in the absence of *IL-10* and *PD-1/PD-L1* (knockout mice), supporting a negative regulatory role for these pathways in M-MDSC activation (53). Furthermore, this retroviral system highlights a reciprocal negative regulation between M-MDSC and Tregs. $CD4^+$ $FoxP3^+$ Tregs from LP-BM5-infected mice were potent producers of IL-10, which was inhibited by MDSCs (114). Conversely, mice depleted of natural $FoxP3^+$ Treg (nTregs), using an adoptive transfer model, had increased M-MDSC frequencies and increased M-MDSC suppressive capacity selectively for T cell responses, compared to nondepleted LP-BM5-infected control mice (114). These results indicated that $CD4^+$ $FoxP3^+$ Tregs normally decreased M-MDSC expansion and activation in LP-BM5-infected mice (either directly or through their effects on cytokine secretion and functions of other immune cells), potentially in an IL-10-dependent manner.

IL-10 was also important for reducing HSK disease severity, but here, $CD11b^+GR1^+$ cells were the major producers of corneal IL-10 (131). $IL-10^{-/-}$ mice with HSK disease had increased disease driven by corneal T cell production of IFN-γ and IL-17 (131), indicating that IL-10 from $CD11b^+GR1^+$ cells may normally act to control HSK disease caused by such T cell responses. Results in Treg-depleted HSK mice supported a role for Tregs as important positive regulators of MDSC recruitment and function during HSV corneal infection (131).

Factors contributing to MDSC recruitment/trafficking

Several molecules involved in the accumulation and expansion have also been shown to play important roles in MDSC trafficking in tumor systems. For example, in addition to its role in preventing DC maturation, S100A9 stimulated recruitment of MDSCs (142). PGE2, another factor that decreased DC maturation, induced expression of CXCR4 and its ligand, CXCL12, in a COX2-dependent manner, which drove accumulation of MDSC at tumor sites (38,111). TGF-β also upregulated CXCR4 on MDSCs, resulting in increased recruitment to tumors, through microRNA-494 (6,91,168). Similarly, hypoxia was shown to increase CCL26 expression on cancer cells, leading to recruitment of CX_3CR1-expressing MDSCs (22).

In some tumor systems, CCL2-dependent attraction of $CCR2^+$ cells, including some TAMs and MDSC subsets, was required for recruitment to tumor sites (16,67). CCR2-dependent pathways were also required for recruitment of MDSC-LCs ($GR1^{lo}Ly6C^{hi}CD11b^+$ and $GR1^{int}Ly6C^{hi}CD11b^+$, respectively) during MCMV and LCMV infections (30,109). Despite established MCMV infection and progression to latency, viral clearance was enhanced in $Ccr2^{-/-}Ccl2^{-/-}$ mice, which exhibit impaired MDSC recruitment (30), implying that MDSCs inhibit immune responses contributing to MCMV clearance.

Pathogen-associated molecular patterns and cytokines can also affect MDSC recruitment. Mortality and MDSC infiltrates to the lung increased in the absence of TLR7 in IAV-infected mice (72), suggesting that in wild-type mice downstream TLR7 signaling events may limit MDSC recruitment and dampen immune responses associated with disease pathology. Supporting such an inhibitory role for TLR7/8 ligation, M-MDSCs from cancer patients differentiated into M1-like macrophages and displayed decreased immunosuppressive capabilities when treated with TLR7/8 agonists (157). Primary hepatic stellate cells, in contrast, promoted G-MDSC expansion *in vitro* and may have supported the recruitment of G-MDSCs to the liver of chronically HBV-infected patients (116). Innate immune sensing of viruses, and the downstream signaling via these pathways, may thus contribute to determining MDSC frequencies during viral infections.

Collectively, these studies in viral systems highlight and implicate several factors involved during MDSC recruitment and/or activation during viral infection: (1) viral sensors and their downstream signaling pathways, (2) viral proteins that normally alter adaptive and/or innate immune responses, and (3) virus-induced cytokines and/or chemokines.

*Phenotype and functional M-MDSC subsets
and dynamic interactions of M-MDSCs with other
immune cells: LP-BM5 retroviral pathogenesis*

As described above, in the LP-BM5 retrovirus MAIDS model, it was determined that M-MDSCs derived from infected mice suppressed both T and B cell responses (Fig. 1a, b) (53). Suppression of T cell responses by LP-BM5-expanded M-MDSCs was iNOS dependent, however, only part of the suppression of B cell responses was dependent on iNOS/NO (53).

VISTA, a newly identified negative checkpoint regulator ligand, expressed on several hematopoietic cell types, including myeloid lineages cells (158), was expressed on M-MDSCs from LP-BM5-infected mice (54). Suppression of B cell, but not T cell, responses by M-MDSCs from LP-BM5-infected mice was partially blocked by the addition of blocking anti-VISTA mAb (54). Use of iNOS and VISTA blocking reagents in combination revealed that the VISTA and iNOS/NO pathways were distinguishable and were the two primary effector mechanisms required for suppression by M-MDSCs (54). In support of (in part) a direct effector suppressive mechanism, a VISTA-Ig chimeric fusion protein also inhibited B cell responses (54).

In addition, soluble mediators, including TGF-β, superoxide anion, peroxynitrite, and NO, contributed to the suppression of B cell responses by LP-BM5-derived M-MDSCs (124) (Fig. 2). However, there was no evidence for involvement of either H_2O_2 or cysteine depletion (124).

M-MDSCs from LP-BM5 retrovirus-infected mice were sorted into several populations and subpopulations, as delineated by the cell surface densities of CD11b and/or Ly6C expression, and these M-MDSC subsets revealed differential suppression of T versus B cell responsiveness (Fig. 1b) (112). The Ly6C$^{+/hi}$ M-MDSC subpopulation was highly suppressive of both T and B cell responses, and suppression was mediated completely or, in part, respectively, by iNOS/NO (112). In contrast, suppression of B cell responses by the Ly6C$^{+/mid}$ M-MDSC subpopulation was undetectable, although this same subset of M-MDSCs readily suppressed T cell responsiveness (112). Consistent with this selectivity for T cell targets, the suppression of T cell responses by this Ly6C$^{+/mid}$ M-MDSC subpopulation was iNOS/NO independent (112), however, the mechanism(s) used to selectively inhibit T cell responsiveness is yet to be identified.

Following LP-BM5 infection and using *in vivo* adoptive transfers of selective CD4$^+$ T cell subsets, it was observed that CD4$^+$ FoxP3$^+$ natural Tregs modulated both the phenotype and function of the M-MDSC population. The suppressive activity toward T cells was significantly increased when M-MDSCs were obtained from nTreg-depleted, LP-BM5-infected mice, compared to M-MDSCs derived from nTreg-intact mice (114). In contrast, suppression of B cell responses was unaffected by nTreg depletion (114). The Ly6C^{mid} population selectively expanded in the absence of nTregs. As just described with sorted subsets in nTreg-intact mice, this subpopulation suppressed T but not B cell responses in an iNOS/NO-independent manner (112,114).

The mechanism by which FoxP3$^+$ Tregs downregulated the LP-BM5-derived M-MDSC compartment *in vivo* remains to be determined, however, studies from this and other systems suggest that IL-10 could be a potential can-didate (53,114). IL-10 can induce Arg1 expression and conversely suppress iNOS expression (56,100). Therefore, early IL-10 production by Tregs, and potentially other cell types, during LP-BM5 infection may decrease iNOS expression in M-MDSCs. LP-BM5-induced M-MDSCs suppressed *in vitro* IL-10 production by both FoxP3$^+$ Treg (114) and regulatory B cells (124), consistent with a reciprocal negative relationship between M-MDSCs and regulatory lymphocytes during LP-BM5 infection.

Direct targeting of MDSCs *in vivo*

The LP-BM5 system demonstrates the complex involvement of MDSCs in disease pathology and indicates potential complications that may arise when using immunotherapies targeting MDSCs. Despite these obstacles, several approaches have been attempted, with varying degrees of success, to target MDSCs in tumor systems (47,117). Although (to our knowledge) the effects of MDSC-targeted therapies in viral infections have not been substantially investigated, these methods may also be useful in targeting MDSCs in infection models, as follows.

MDSCs as therapeutic targets during viral infection. In many viral diseases, MDSC numbers correlated with pathogenesis, including in HIV (24,25,122,155), LP-BM5 (53), and more than half of the reported studies in HCV (14,52,170). Conversely, antiviral therapies, which decrease pathology, were associated with decreased MDSC numbers in HCV (14) and HIV/SIV (49,122,155). One of the roles of MDSCs in pathogenesis is to decrease antiviral immunity, possibly to limit immunopathological damage. Expansion of MDSCs is observed in chronic, but not acute LCMV, indicating that MDSCs may contribute to the establishment of chronic infection (109).

In several models where MDSCs were intentionally reduced, antiviral responses and viral clearance increased, including via anti-GR-1 mAb therapy for LCMV (109) and HCV (164). MCMV-infected *CCR2/CCL2* double knockout mice, which had impaired MDSC recruitment, also exhibited increased viral clearance (30). SIV vaccines using MVA virus as a vector and adjuvant resulted in increased MDSC frequency when compared to vaccination with adjuvant alone (146). This increase in MDSCs was accompanied by increased viral load, also implicating MDSCs in suppressing antiviral immunity (146). In these virus infections where MDSCs decrease protective antiviral responses, immune therapies aimed at decreasing MDSC numbers or function may aid in viral clearance and prevent chronic infection.

On the contrary, MDSCs show evidence of being protective to damage sustained in certain viral infections. During vaccinia infection, depletion of MDSCs with anti-Gr-1 led to increased mortality and was attributed to increased NK cell activity and IFN-γ production, indicating the MDSC may prevent lethal immunopathology (42). Similarly, in HBV-infected patients, decreased MDSC frequencies correlated with hepatic flares (116). In viruses that cause severe immunopathology, increasing MDSC numbers may prove beneficial. Robust MDSC function may also benefit the host by decreasing the number of other cell types available for productive infection, especially in cases such as HIV (122) and LP-BM5 (59), when immune cells are targets for infection and are required for pathogenesis.

In yet other cases, evidence indicates that MDSCs may play dual roles—being both harmful to the host (preventing antiviral immunity) and beneficial (preventing immunopathology). Kit^{W-sh} mice (which lack CD31$^+$ mast cells) infected with HSV-1 had increased G-MDSC recruitment to the cornea, and increased HSK and viral titers, indicating that the G-MDSCs may dampen HSV-1 antiviral responses (130). However, IL-10 is important for reducing HSV-1-induced immunopathology in the cornea, and MDSCs are the major source of IL-10 production (131). Immunotherapy targeting MDSCs may be helpful in cases where one of these functions needs to be increased or decreased.

Blocking MDSC expansion/generation.   There are a variety of pharmacological approaches that may be able to block expansion or generation of MDSCs *in vivo*. For example, tasquinimod binds to and inhibits signaling by S100A9, a molecule known to be important for accumulation and activation of MDSCs (126). In murine prostate tumor models, tasquinimod treatment led to decreased MDSC numbers and functions, and increased antitumor responses (137). This treatment was well tolerated, slowed tumor progression, and increased survival in phase II trials in castrate-resistant prostate cancer patients (119). These data indicate that tasquinimod may also be a beneficial therapy for viral infections, in which S100A9 signaling is involved in MDSC expansion.

As mentioned above, the PGE2-COX2 pathway has also been implicated in MDSC generation (110,111). COX2 inhibitors have been tested in a variety of cancers and have reduced tumor growth, possibly due, in part, to decreased MDSC generation (17,156,160). Conversely, COX2 promoters, such as the FDA-approved drug finasteride, were able to increase MDSC numbers and may be useful when immunosuppressive therapy is desired (172).

Ablating TNF-α, another factor involved in MDSC induction, using the clinically approved TNF blockers etanercept and infliximab, was shown to reduce MDSC accumulation and transplantable tumor growth in mice with the human TNF-α gene substituted for the murine gene (1). The clinically approved cancer therapeutics ibrutinib and sunitinib inhibited tyrosine kinases present in MDSCs and prevented MDSC generation, migration, and function in several murine tumor models (64,145).

Inducing MDSC differentiation and maturation.   As MDSCs are IMC populations that include precursors of macrophages and myeloid DCs, one potential therapeutic strategy is to induce differentiation of these cells to more mature, nonsuppressive DCs and macrophages (47): for example, using all-trans retinoic acid (ATRA) (45,61,78). ATRA treatment successfully increased T cell responsiveness and decreased MDSC numbers (with MDSCs differentiating into DCs, macrophages, and a small number of granulocytes) in a variety of murine tumor models (78). These changes were found to be independent of the effects of ATRA on tumor cells, indicating that this therapy could potentially be used in viral systems (78). Clinical trials in patients with lung cancer have verified that ATRA decreased MDSC numbers and increased p53-expressing DC vaccine efficacy (70). In a murine model of postsepsis immunosuppression, ATRA administration concurrently resulted in decreased MDSC numbers and increased CD4$^+$ and CD8$^+$ T cell numbers (96).

MDSCs can also be differentiated into mature myeloid cells using paclitaxel or potent stimulation by pathogen-associated molecular patterns, such as CpG oligonucleotides (97,139). Cryothermal therapy, in which cooling and heating are alternated, has been shown to lead to release of Hsp70, which induced differentiation of MDSCs into mature DCs, allowing for increased survival and decreased metastasis in a murine mammary carcinoma model (175).

Depleting MDSCs.   In the Theiler's murine encephalomyelitis virus disease model, deletion of GR1$^+$ MDSCs with the anti-GR1 antibody delayed onset and reduced clinical development of demyelinating disease (7). Polyfunctional LCMV-specific T cells increased coordinately with anti-GR-1-mediated diminishment of MDSCs in LCMV-infected mice, with a trend toward decreased viral loads (109). However, MDSC reduction during vaccinia virus infection in mice instead led to increased mortality and was associated with increased NK cell activation and IFN-γ inflammatory responses, rather than increased viral titers (42).

The anti-GR1 antibody binds both granulocytic and monocytic MDSC subsets, but can also bind monocytes and mature PMNs, leading to broad myeloid cell depletion. Such broad targeting could have deleterious effects, particularly if non-MDSC targets, or one of the subsets of MDSCs, are beneficial to viral clearance or prevention of immunopathology. In an HSV-1 skin flank infection model, mice depleted of GR1$^+$ cells had increased viral loads and lesion severity. This effect was not observed in mice depleted of Ly6G$^+$ cells (including neutrophils and G-MDSCs) (164), suggesting that Ly6C$^+$ GR1$^+$ cells (M-MDSCs) may modulate the immune response needed to control HSV-1 viral replication and associated immune pathology. A similar pattern of an inferred role for Ly6C$^+$ M-MDSCs was observed by the same research group using an intranasal HSV-1 infection model (165).

Administration of the chemotherapeutic agent gemcitabine, a nucleoside analog that lowers MDSC numbers in the spleen by inducing apoptosis and necrosis, decreased MDSCs in murine tumor models (37,80,147).

Blocking suppressive mechanisms.   Blocking the suppressive mechanism(s) of MDSCs could be an attractive alternative to MDSC depletion. Specific inhibitors exist to block Arg1, iNOS, and ROS production. For example, nitroaspirin has been found to limit Arg1 and iNOS activity in splenic MDSCs (32). Phosphodiesterase-5 inhibitors such as sildenafil, tadalafil, and vardenafil, which limit Arg1 and iNOS activity, have been shown to restore T cell antitumor immunity in murine models and in a patients with end-stage relapsed/refractory multiple myeloma (108,134).

However, the plasticity of MDSCs and the variety of potential suppressive mechanisms may allow them to compensate, when certain mechanisms are blocked, by upregulating other mechanisms. Such compensation was observed with comprehensive mechanistic studies, including wild-type versus *iNOS/VISTA* double knockout MDSCs in the LP-BM5 murine retrovirus model (124). This plasticity and the observation that MDSCs may use multiple suppressive mechanisms (Figs. 1a and 2) indicate that inhibition of certain mechanisms may not have as profound an effect as blocking expansion, inducing differentiation, or depleting MDSCs.

Vaccination. MDSC immune responses were altered following some vaccination regimens. As mentioned above, in an SIV vaccination study, increased frequencies of MDSCs, correlating with increased viral loads, were observed in animals treated with MVA vector plus adjuvant-vaccinated compared to the adjuvant-alone group (146). The MVA vector vaccine-induced MDSCs inhibited protective immune responses, leading to decreased vaccine efficacy (146). Decreased CD8^{+} CTL activity attributed to increased MDSCs was also detected in mice immunized with an infectious recombinant vaccinia virus encoding IL-2 (11). CD11b^{+}Ly6C^{int}Ly6G^{−} M-MDSCs accumulated at the site of bacillus Calmette–Guérin (BCG) vaccination and were associated with dampened early T cell priming responses (95).

Other viruses commonly used in vaccinations as vectors and as recombinants, such as vesicular stomatitis virus, have also been shown to induce MDSC accumulation (42,90). Certain adjuvants, including the dsRNA synthetic analog poly(I:C) (90), complete Freund adjuvant (161), and the lipopolysaccharide-derived TLR4 agonist monophosphoryl lipid A (19), also induced MDSC expansion. Conversely, the TLR7/8 antagonist, resiquimod, caused MDSC differentiation into less suppressive, mature myeloid cells (84). Conflicting reports indicate that CpG may either expand MDSCs (18,99) or increase their differentiation into macrophages (139).

Effects on microbial pathogenesis

In addition to suppressing immune cells, MDSCs can directly alter microbial pathogenesis. For example, NO is known to have antimicrobial and antiparasitic activity (71,102). In the context of viral infections, NO production or arginine depletion can alter/inhibit viral replication, cause immunopathology, and/or promote viral persistence, in part, by modifying innate and/or adaptive immune responses (13).

Relative to the mechanism(s) utilized by MDSCs to suppress immune cells during viral infection, some of these molecular mechanisms may have diverse "off-target" effects—that is, on the virus itself, nonimmune cells, or immune cells unassociated with protection. While blocking an MDSC suppressive mechanism(s) may lead to compensatory inhibitory mechanisms, even direct depletion could result in unexpected and undesired consequences such as upregulation of other inhibitory cells (e.g., IL-10-producing CD4^{+} Treg cells). This consequence is consistent with the observation that during LP-BM5 retrovirus infection, Tregs are normally either directly or indirectly suppressed by MDSCs (114).

Conclusions and Perspectives

Several of the studies exploring MDSCs in patients consist of small sample sizes, which limit the interpretation of results. In addition, MDSCs isolated *ex vivo* from fresh or frozen human PBMC samples, or derived under potentially non-physiological *in vitro* conditions, may alter the phenotype and/or functionality of these MDSCs. These difficulties in human systems make murine studies an attractive alternative. However, confounding factors, such as the disparate use of MDSC markers, make comparing human and murine studies challenging. Additional studies are needed to fully understand differences between murine and human MDSCs and to more effectively design safe and beneficial MDSC-related therapies.

While MDSCs are subdivided into monocytic and granulocytic subsets, these cell compartments remain highly heterogeneous. Phenotypic characteristics used to identify MDSCs are similar to other immune cells, including neutrophils, DCs, monocytes, and macrophages. Suppressive mechanisms may vary in different infections, with many MDSC populations/subpopulations utilizing multiple mechanisms. Inhibition of one suppressive mechanism may lead to upregulation of other mechanisms. In comparison to the heavily studied role of MDSCs in cancer [reviewed in Refs. (101,150)], their roles in viral infections remain relatively uncertain. However, there is growing enthusiasm that sufficient similarities in MDSCs exist across many different pathologies to allow for more broad therapies targeting MDSCs to attain a better balance of antiviral immunity versus immunopathology.

Author Disclosure Statement

No competing financial interests exist.

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