MYELOID DERIVED SUPPRESSOR CELLS AS DISEASE MODULATORS

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MYELOID DERIVED SUPPRESSOR CELLS AS DISEASE MODULATORS

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Myeloid Derived Suppressor Cells (MDSCs) are a heterogeneous population of immature myeloid cells that can suppress the function of multiple immune cells and in particular, T cells, through various mechanisms. MDSCs can be divided into two major subtypes based on their cell surface phenotype and morphology: polymorphonuclear MDSC (PMN-MDSC or G-MDSC) and monocytic MDSC (M-MDSC). Additional subtypes have been proposed, such as the early MDSC (e-MDSC) that lack both macrophage and granulocyte markers. There is still considerable ambiguity about the phenotype of these cells that corresponds to their immunosuppressive function and there are on-going challenges on how to identify, purify and/or potentially generate and expand these cells in vitro. MDSCs were first discovered in cancer patients where they have been most extensively studied as components of the immunosuppressive tumor microenvironment. In the last several years, however, the importance of their immunomodulatory role in many other disease and clinical settings has emerged.

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EDITORIAL
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Editorial: Myeloid Derived Suppressor Cells as Disease Modulators

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Keywords: myeloid-derived suppressor cells (MDSC), cancer, immunosuppression, arginase 1 (Arg-1), chronic inflammatory diseases

Editorial on the Research Topic

Myeloid Derived Suppressor Cells as Disease Modulators

Myeloid cells are a diverse family of innate immune cells with enormous functional plasticity stemming in part from the lack of genetically encoded antigen-specific receptors. Monocytes, dendritic cells and the various forms of polymorphonuclear granulocytes (eosinophils, basophils, and neutrophils) play fundamental roles in our defense against infectious agents. However, in chronic inflammatory conditions such as cancer, chronic infections, obesity, trauma and chronic stress, myeloid cells become chronically activated, develop mechanisms that suppress T cell, B cell, and even NK cell functions and have thus been named myeloid-derived suppressor cells (MDSC) (1). Similar to their normal counterparts MDSC can be monocytic (M-MDSC) or granulocytic (PMN or G-MDSC) and display a wide array of immunosuppressive mechanisms (2–4). In cancer, where they have been most extensively studied, MDSC can be detected early on in the malignant microenvironment (5) and increase in circulation as the tumors progress. Increases in the numbers of circulating MDSC have been associated with a decreased response to check-point immunotherapies and poor overall survival (6, 7).

The signals and mechanisms that activate and regulate normal myeloid cell function are primarily pathogen-associated molecular patterns (PAMPs) from infectious agents and damage-associated molecular patterns (DAMP's) from damaged tissues. The elimination of the infectious agent or the repair of tissues ends the response of myeloid cells which return to a quiescent stage. In contrast, diseases characterized by chronic inflammation and/or persistent tissue damage such as cancer, autoimmunity, or chronic infections, result in the prolonged release of DAMP's and PAMP's and the production of cytokines such as G-CSF, GM-CSF, and IL6 that increase the release of myeloid-cells from bone marrow and promote the induction of immunosuppressive mechanisms in MDSC. More recently new data show that increased concentrations of lipids such as found in obese patients (8, 9), or increased levels of catecholamines as in chronic pain or stress also promote the activation of immunosuppressive mechanisms by MDSC (10). MDSC suppress T and NK cell function through multiple mechanisms. The depletion of amino-acids such as arginine and L-tryptophan by Arginase I and Indoleamine 2,3-dioxygenase (IDO) induces T cell anergy, while an increased uptake of cysteine by MDSC depletes this amino-acid that is essential for T cell function. The production of reactive oxygen species (ROS) and reactive nitrogen species (nitric oxide—NO) induces T cell apoptosis, while the release of immunosuppressive cytokines such as IL10 and TGFβ, or the production of adenosine inhibit T and NK cell functions. Finally the expression of check-point molecules such as PD-L1 leads to T cell exhaustion, while Fas L and Galectin 9

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cause T cell apoptosis. The end result is the loss of protective or therapeutic T cell responses and the escape of tumors from the immune response or the therapeutic effect of novel immunotherapies.

MDSC are therefore the focus of intense research aimed at identifying signals that increase and activate MDSC, understanding their role in different diseases, establishing unique markers that allow us to track the number and fate of these cells, and finding therapeutic approaches to block their immunosuppressive activities. The publications that are part of the series on Myeloid Derived Suppressor Cells as Disease Modulators present original articles and reviews that update on the recently acquired knowledge of the mechanisms involved in the induction and function of MDSC in cancer and other diseases and discuss therapeutic approaches being tested for modulating their function with the goal of allowing the development of a protective T cell functions that resolve the disease process.

AUTHOR'S NOTE

The authors selected and invited the scientific contributors to this collection based on their unique and pioneering discoveries on the role of MDSC in a variety of diseases, the biology of MDSC, their impact on the function of other immune cells and their effect on disease outcomes. We expect that the knowledge presented in these articles provides information for other researchers in the field and eventually helps develop novel therapeutic approaches to regulate the function of MDSC for the benefit of patients.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
2. Ostrand-Rosenberg S, Fenselau C. Myeloid-derived suppressor cells: immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J Immunol.* (2018) 200:422–31. doi: 10.4049/jimmunol.1701019
3. Si Y, Merz SF, Jansen P, Wang B, Bruderek K, Altenhoff P, et al. Mutlidimensional imaging provides evidence for down-regulation of T cell effector function by MDSC in human cancer tissue. *Sci Immunol.* (2019) 4:eaaw9159. doi: 10.1126/sciimmunol.aaw9159
4. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer.* (2019) 120:16–25. doi: 10.1038/s41416-018-0333-1
5. Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. *Cancer Prev Res.* (2013) 6:18–26. doi: 10.1158/1940-6207.CAPR-12-0275
6. Ai L, Mu S, Wang Y, Wang H, Cai L, Li W, et al. Prognostic role of myeloid-derived suppressor cells in cancers: a systematic review and meta-analysis. *BMC Cancer.* (2018) 18:1220. doi: 10.1186/s12885-018-5086-y
7. Wang PF, Song SY, Wang TJ, Ji WJ, Li SW, Liu N, et al. Prognostic role of pretreatment circulating MDSCs in patients with solid malignancies: a meta-analysis of 40 studies. *Oncoimmunology.* (2018) 7:e1494113. doi: 10.1080/2162402X.2018.1494113
8. Ostrand-Rosenberg S. Myeloid derived-suppressor cells: their role in cancer and obesity. *Curr Opin Immunol.* (2018) 51:68–75. doi: 10.1016/j.coi.2018.03.007
9. Al-Khami AA, Rodriguez PC, Ochoa AC. Metabolic reprogramming of myeloid-derived suppressor cells (MDSC) in cancer, *Oncoimmunology*. (2016) 5:e1200771. doi: 10.1080/2162402X.2016.1200771
10. Repasky EA, Eng J, Hylander BL. Stress, metabolism and cancer: integrated pathways contributing to immune suppression. *Cancer J.* (2015) 21:97–103. doi: 10.1097/PPO.0000000000000107

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REVIEW
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Reactive Oxygen Species as Regulators of MDSC-Mediated Immune Suppression

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Reactive oxygen species (ROS) molecules are implicated in signal transduction pathways and thereby control a range of biological activities. Immune cells are constantly confronted with ROS molecules under both physiologic and pathogenic conditions. Myeloid-derived suppressor cells (MDSCs) are immunosuppressive, immature myeloid cells and serve as major regulators of pathogenic and inflammatory immune responses. In addition to their own release of ROS, MDSCs often arise in oxidative-stress prone environments such as in tumors or during inflammation and infection. This evidently close relationship between MDSCs and ROS prompted us to summarize what is currently known about ROS signaling within MDSCs and to elucidate how MDSCs use ROS to modulate other immune cells. ROS not only activate anti-oxidative pathways but also induce transcriptional programs that regulate the fate and function of MDSCs. Furthermore, MDSCs release ROS molecules as part of a major mechanism to suppress T cell responses. Targeting redox-regulation of MDSCs thus presents a promising approach to cancer therapy and the role of redox-signaling in MDSCs in other disease states such as infection, inflammation and autoimmunity would appear to be well worth investigating.

Keywords: ROS, MDSC, Nrf2, redox regulation, metabolism

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INTRODUCTION

Reactive oxygen species (ROS) appear to have harmful as well as beneficial effects(1, 2). Their harmful effects include oxidation-induced damage to cellular contents, such as lipids, proteins, carbohydrates and nucleic acids, which subsequently induce cell pathologies and cell death. Damaged and oxidized molecules contribute to a number of alterations including atherosclerosis, neurodegenerative diseases and aging. Beyond this, ROS molecules are implicated in signal transduction pathways and redox-dependent regulations controlling a range of biological activities. In this regard, it is interesting to examine myeloid-derived suppressor cells (MDSCs). These heterogenic myeloid cells are controlled by ROS but they also use ROS to fulfill suppressive functions. Pathological conditions such as chronic inflammation, infection and cancer, induce MDSCs, which consist of a heterogeneous population of immature myeloid cells (3, 4). A hallmark of these immunosuppressive cells is their capability to suppress T cell responses, which contributes to cancer immune evasion on the one hand but suppression of exaggerated T cell responses during inflammation on the other. In mice, MDSCs are broadly characterized by the surface expression of CD11b and Gr-1 and are further grouped into monocytic ($CD11b^{+}Ly6C^{high}Ly6G^{-}$) and polymorphonuclear ($CD11b^{+}Ly6C^{low}Ly6G^{+}$) MDSCs

(5). The polymorphonuclear (PMN-MDSC) subset displays increased STAT3 and NADPH oxidase (Nox) activity, which results in high release of ROS but low NO release. The monocytic subset (M-MDSC) express high levels of STAT1 and iNOS and enhanced level of NO but show low ROS production. Both of them express arginase 1 (3). Although ROS have toxic effects on most cells, MDSCs survive despite elevated levels and continuous production of ROS (6). ROS production is not only central to the immunosuppressive properties of MDSCs but also seems to maintain them in an undifferentiated state. Furthermore, steady-state production of ROS by MDSCs is upregulated in a variety of murine tumor models and in human cancer, and also after activation in inflammatory and autoimmune conditions (4, 7). In addition to their own ROS release, MDSCs often arise in oxidative-stress prone environments such as in tumors or during inflammation and infection. This evidently close relationship between MDSCs and ROS prompted us to summarize what is currently known about ROS signaling in MDSCs itself and to elucidate how MDSCs use ROS to modulate other immune cells.

MAIN TEXT

Regulation of MDSCs by ROS

A state of "oxidative stress" describes a situation where high levels of ROS -derived from cellular metabolism, toxic insults, or oxidative burst- outbalance the anti-oxidative system (8). This breakdown of cellular homeostasis results from mitochondrial dysfunction or increased metabolic activity, oncogene activity or infiltrating immune cells (8) and induces damage to lipids, proteins, carbohydrates and nucleic acids and can even lead to cell death (9). Excessive production of ROS molecules is associated with several inflammatory and pathologic conditions. For example, oxidative stress within the intestinal epithelium is thought to be involved in the pathogenesis of intestinal inflammation (10) and oxidative stress is also associated with neurodegenerative diseases (8). Furthermore, elevated rates of ROS can be observed in almost all cancers and are involved in tumor metastasis (11). On the other hand, emerging evidence suggests that ROS molecules serve as signaling intermediates that play central roles in several molecular pathways and also serve as central mediators of immune cells (12). Low levels of ROS are continuously generated under healthy cellular conditions, and are neutralized by the endogenous antioxidant machinery that is regulated by nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 is retained and degraded in the cytosol by Kelch ECH associating protein 1 (Keap1) under basal conditions (13). Cellular stimuli such as oxidative stress lead to conformational changes in Keap1, which are followed by the release of Nrf2 from Keap1. Afterwards, Nrf2 translocates into the nucleus, where transactivation of genes containing an antioxidant response element (ARE) in their promoter regions takes place (14). Thereby, Nrf2 up-regulates phase II detoxifying enzymes and antioxidant proteins. These processes play a vital role in maintaining cellular homeostasis and are of major relevance upon exposure of cells to chemical or oxidative stress and inflammation. Particulary enzymes mediating gluathione (GSH) synthesis, the thioredoxin enzyme system, and detoxifying enzymes such as heme oxygenases, or NAD(P)H: quinone oxidoreductase 1 are part of the Nrf2 induced enzymatic machinery.

The most prevalent intracellular sources of ROS are mitochondria and NADPH oxidases (Nox) but beyond this, the ER and also the peroxisome (organelle that metabolizes long chain fatty acids) generate ROS molecules. Nox-mediated release of ROS induces the so-called oxidative burst and eliminates invading microorganisms (15). The relevance of Nox-derived ROS in host immunity is best demonstrated by the disease pattern of chronic granulomatous disease (GCD), which is caused by NOX2 defects, and results in hypersensitivity to common infections and accumulation of bacteria-containing phagocytes with subsequent granulome development (15, 16).

Mitochondrial ROS are central regulators of the innate immune system. They are indispensable for Toll-like receptor (TLR)-initiated pathways (17). In detail TLR1, TLR2, and TLR4 signaling leads to recruitment of mitochondria to phagosomes and enhances ROS production in macrophages, indicating that mitochondrial ROS form an important component of antibacterial responses and are necessary for activation of NLRP3 inflammasome (18). In addition to this, mitochondrial ROS are involved in NLRP3 activation. Accumulation of damaged ROS-generating mitochondria leads to NLRP3 activation (19), and increased levels of mitochondrial ROS resulting from NLRP3 activation serve as a feedback mechanism to sustain activation (20).

Furthermore, mitochondrial ROS and ROS derived from other sources and cellular metabolism are intimately linked. Oxidative phosphorylation (OXPHOS) is a major cellular source of ROS and requires adequate availability of antioxidants to prevent apoptosis. One advantage of glycolysis over OXPHOS lies therefore in a better maintenance of the redox balance. Lian et al. recently observed that MDSCs counteract OXPHOS-derived ROS by upregulation of glycolysis, thereby protecting MDSCs from apoptosis (**Figure 1**) (23). We observed high OXPHOS in MDSCs of mice with a constitutive Nrf2 activation and subsequently low levels of intracellular ROS (22). The constitutive activation and availability of antioxidant enzymes regulated by Nrf2 activation in these cells might be a central mechanism enabling the cells to increase mitochondrial ATP production by simultaneously counteracting subsequent high ROS levels. High oxygen consumption rate (OCR) levels were associated with a highly suppressive and tolerizing phenotype. Recent studies have shown that aerobic glycolysis constitutes the metabolic basis for trained immunity (24). The metabolism of tolerant myeloid cells, particularly of MDSCs, is less clearly understood and was one focus of our study (22). It is generally assumed that naïve or tolerant cells primarily use

Abbreviations: ATRA, All-trans retinoic acid; CDDO-Me, C-28 methyl ester of 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid; Cys, cysteine; GSH, Glutathione; HCC, Hepatocellular carcinoma; HIF, Hypoxia-inducible factor; Keap1, Kelch ECH associating protein 1; MDSC, Myeloid-derived suppressor cell; M-MDSC, monocytic MDSC; NOX, NADPH oxidase; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; OXPHOS, oxidative phosphorylation; PMN-MDSC, polymorphonuclear MDSC; PPP,pentose phosphate pathway; ROS, reactive oxygen species.

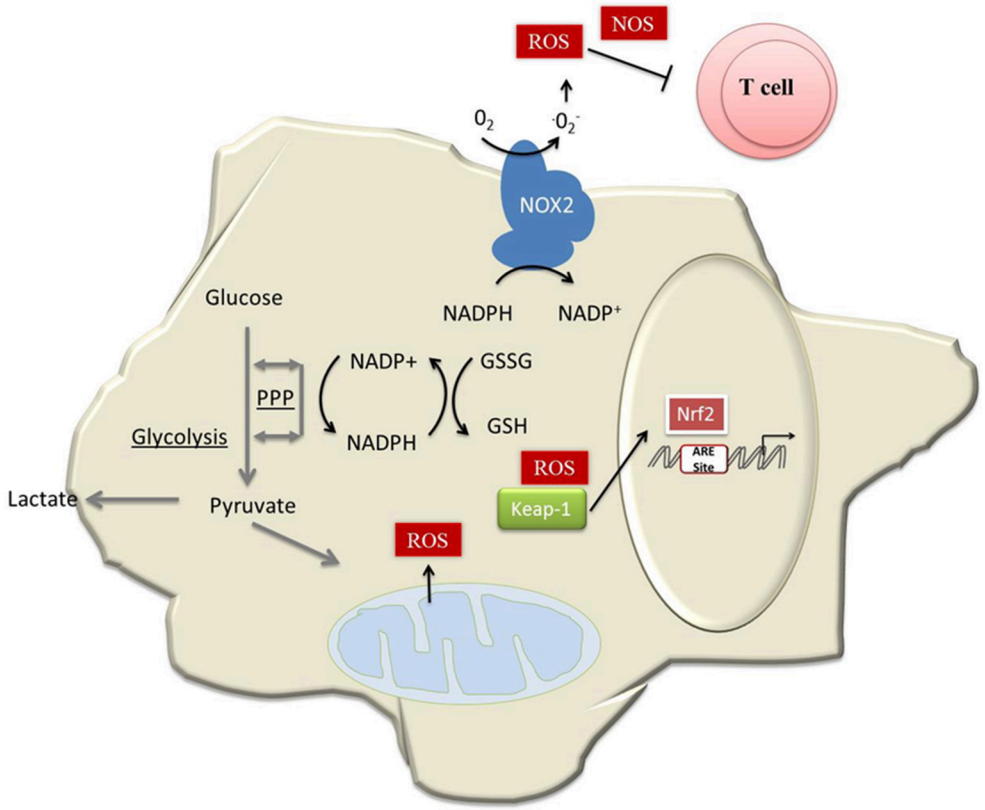

FIGURE 1 | Model of how MDSCs maintain redox homeostasis. Activated MDSCs produce high amounts of ROS molecules by the action of NOX2 (4). This, in addition to mitochondrial ROS and ROS derived from cancer cells, compromises redox homeostasis in MDSCs and most likely induces apoptosis in the absence of Nrf2 (21). Another consequence of Nrf2 activation, besides expression of antioxidative genes, is a metabolic reprogramming of MDSCs. This leads to enhanced expression of the PPP (22), which provides GSH. GSH not only serves as a major antioxidant, but is essential for MDSC differentiation (22). In addition, MDSCs counteract OXPHOS-derived ROS by upregulation of glycolysis (23).

OXPHOS as an energy source, but activated cells, e.g., after LPS stimulation, undergo a shift toward aerobic glycolysis (25). However, metabolic characteristics of MDSCs seem to differ within this quite heterogeneous cell population and might also depend on disease context. In comparison to splenic MDSCs, tumor-infiltrating MDSCs enhance fatty acid oxidation (26). However, rapamycin, which specifically inhibis mTOR, reduces M-MDSC in mice with allografts or tumors (27). Flux of glucose down the pentose phosphate pathway (PPP) is essential for redox buffering as PPP produces NADPH. This is required to maintain GSH, the most important cellular antioxidant, in the reduced state (**Figure 1**). Again, we observed a high expression of PPP enzymes in Nrf2-activated MDSCs, which suggests that Nrf2 is critically involved in redox and in the metabolic signaling of MDSCs, and acts either by mediating ROS signaling or possibly also by targeting other genes (22).

Redox signaling is moreover involved in several signal transduction pathways. In most cases cystein (Cys) residues serve as redox-dependent switches and the oxidation/reduction of specific amino acids, that bear reactive Cys residues, induces activation, or inactivation of target proteins such as phosphatases. Moreover, ROS modulate antioxidant enzymes that not only serve as scavengers but also transduce redox-dependent signals (28). GSH is not only the most important antioxidant in cells in general but also mediates specific effects in immune cells. For instance, GSH is involved in reprogramming of effector T cells during inflammation (29). With regard to MDSCs, increased levels of GSH are especially important for MDSC differentiation (30). Probably, GSH affects MDSCs differentiation by neutralization of ROS but other direct effects of GSH on MDSCs are conceivable.

ROS molecules are essential for maintainance of MDSCs in their undifferentiated state. Scavenging of H_2O_2 with catalase induces differentiation of immature myeloid cells into macrophages in mice bearing tumors (31), while in the absence of Nox activity, MDSCs differentiate into macrophages and DCs in tumor-bearing mice (4). Increased levels of endogenous H_20_2 might thereby present a mechanism by which tumors prevent the differentiation of MDSCs. The precise molecular mechanism maintaining MDSCs in their undifferentiated state in the presence of ROS remains to be elucidated.

Regulation of Cellular Immune Responses by MDSC-Derived ROS

Release of ROS molecules is one of the major mechanisms that MDSCs use to suppress T cells in mice and humans (4, 32, 33). Administration of ROS inhibitors was found to counteract the suppressive effect of human MDSCs on T cells (34). And, at least in tumor-bearing mice, suppression of T cells is dependent on NOX2 activity (4). Superoxide released by MDSCs rapidly reacts with a large number of molecules e.g., H_2O_2, hydroxyl radical, hypochlorous acid, and peroxynitrite to form ROS, which then damage proteins, lipids, and nucleic acids, enhance inflammation and promote apoptosis. ROS are even thought to enable Ag-specific suppression of T cell responses by MDSCs. Nagaraj et al. showed that MDSC-derived ROS molecules and peroxynitrite, which is the product of the reaction of ROS with NO, modify TCR and CD8 molecules. Through these modifications, $CD8^+$ T cells lose their ability to bind phosphorylated MHC and induce antigen-specific tolerance of peripheral $CD8^+$ T cells (35).

H_20_2, formed from MDSC-derived superoxide, decreases T cellular CD3ζ expression, thereby limits the ability of the T cells to become activated (36) and reduces their expression of IFN-γ (4).

While MDSCs suppress effector T cells, they induce the expansion of regulatory T cells (T_{regs}) in cancer, and also in inflammatory conditions (37–42). Induction of T_{regs} is therefore one important mechanism of MDSC-mediated T cell inhibition. The role of ROS molecules in the interaction of MDSCs and T_{regs} is not clear. The induction of T_{reg} cells by macrophages involves production of ROS and therefore ROS deficiency might lead to reduced T_{reg} induction and might aggravate T-cell suppression (43). In addition, T_{regs}, are less susceptible to oxidative stress-induced cell death compared to other T cell populations (44). This is most likely caused by a greater secretion of redox proteins such as thioredoxin (44) or hemeoxygenase 1 (45). In addition to this, human T_{regs} have been shown to express high levels of cell surface thiols, that are important reducing agents, and facilitate enhanced intracellular anti-oxidative abilities (44). Nevertheless, a recent study claims that T_{reg} cells are less resistant to oxidative stress in the tumor microenvironment compared to conventional T cells and even undergo ROS-induced apoptosis due to a weak Nrf2-associated antioxidant system (46). These apoptotic T_{reg} cells suppress antitumor T cell immunity even more efficiently via the adenosine and A_{2A} pathways. As a consequence, T_{regs} or at least T_{reg}-mediated suppression seems to benefit from oxidative stress conditions and might therefore contribute to MDSC-mediated immune suppression.

Beyond direct effects on T cells, ROS molecules also indirectly modulate T cell responses. Peroxynitrite indirectly hinders T cells activation by modifying the antigen presenting structure on tumor cells. To this end, peroxynitrite reduces the binding of antigens to tumor cell-associated MHC and thereby generates tumor cells that are resistant to antigen-specific cytotoxic T cell responses (47). Furthermore reactive nitrogen species induce posttranslational modifications of T cell chemokines and thereby hinder antigen-specific T cells invasion of tumors (48).

Furthermore, not only T cell responses are targets of ROS mediated suppression by MDSCs. PMN-MDSCs also suppress NK cell responses to adenoviral vectors and to vaccinia virus infection by ROS release (49, 50). In addition, MDSCs also suppress NK cell toxicity in tumor bearing mice and might critically contribute to the attenuated NK cell activity and cytotoxicity in tumors (51), however the exact mechanism and involvement of ROS are not fully determined.

Recent research demonstrates that MDSCs also negatively regulate B cell- mediated immune responses using ROS. In a murine AIDS model (LP-BM5 reotroviral infection) M-MDSC suppressed B cell responses at least in part by ROS mediated suppression (52, 53). Astudy with human PMN-MDSCs demonstrates that MDSCs suppress B cell proliferation and antibody production in a cell contact manner by means of arginase, NO and ROS (54).

REDOX-DEPENDENT TRANSCRIPTIONAL REPROGRAMMING OF MDSCS IN CANCER AND INFLAMMATION

It is of note that HIF-1α and Nrf2, which are both involved in redox-signaling and oxidative stress responses, emerge as critical regulators of MDSCs. Beyond redox regulation; both factors control other mechanisms and thereby regulate MDSC fate and function.

A critical role of HIF-1α signaling in MDSCs is described in murine cancer models, such as hepatocellular carcinoma (HCC) (21, 55, 56) and in patients with non-small cell lung cancer (57). Interestingly, HIF-1α controls the manner of MDSC-mediated suppression, depending on the hypoxic state of the environment. The dominant mechanism in peripheral lymphoid organs is mediated by ROS and results in antigen-specific T cell non-responsiveness. However, within the hypoxic tumor microenvironment, MDSCs bearing the same phenotype and morphology revealed low levels of ROS levels but significantly enhanced NO production as well as arginase activity and thereby suppressed T cells (21). Several mechanisms have been analyzed by which HIF-1α regulates the fate and function of MDSCs in a hypoxic tumor environment. Some of these studies come to contradictory conclusions, possibly due to the use of different tumor models or the heterogeneity of MDSC populations. Liu et al. showed that lineage differentiation of MDSCs to M1 cells requires glycolytic activity induced by mTOR- and HIF-1α, as brought about by SIRT1 in tumors (56), while Cocl2 (an HIF-1α activator) effectively promotes M1-MDSC differentiation, and potentiates tumor-killing and glycolytic activities. On the other hand, HIF-1α was found to upregulate PD-L1 on MDSCs and induce miR-210, both of which enhance MDSC-mediated T cell suppression (58, 59). In conclusion, these studies reveal that by regulating several pathways including metabolic reactions and miRNA expression, HIF-1α critically regulates the function and maintenance of MDSCs within the hypoxic tumor environment.

Nrf2 is involved in the regulation of various pathways in MDSCs as well. Through an analysis of Nrf2-deficient mice in mammary carcinoma and colon carcinoma models,

Beury et al. initially showed that Nrf2 regulates numbers and function of MDSCs (6). Nrf2 deficient mice had increased survival rates and reduced tumor progression with reduced numbers of MDSCs and MDSCs from Nrf2-deficient mice had a reduced suppressive capacity and, surprisingly, a reduced H_20_2 production. Intracellular oxidative stress and apoptosis were enhanced in the absence of Nrf2. However, myeloid-lineage specific Nrf2 deficiency enhances lung metastasis and has been shown to lead to an aberrant ROS accumulation in myeloid cells (60). Nrf2 is known to play dual roles in cancer prevention and progression, which depends on the cellular context and environment (61). However, the exact mechanisms involved remain to be elucidated. It is also not clear whether Nrf2 expression—like HIF-1α expression—is different in peripheral lymphoid organs and tumor MDSCs and whether it might therefore also influence local MDSC maintenance. We observed spontaneously enhanced numbers of MDSCs in mice with a constitutive activation of Nrf2 with intact suppressive functions *in vitro*. This was also found in a transfer colitis model and in a sepsis model *in vivo* (22). MDSCs with constitutive Nrf2 activation displayed low levels of intracellular ROS, but a high metabolic activity and high proliferation rates. This suggests that, beyond its anti-oxidative action, Nrf2 has several other effects that need to be taken into account and might contribute to a context-dependent regulation of MDSCs.

CONCLUSION

ROS signaling is without doubt a central mediator of MDSC function and fate. Furthermore, beyond their role in MDSC-mediated immune-suppression, ROS molecules are intrinsically involved in activation of transcription factors such as Nrf2 and HIF-1α, which can induce transcriptional and metabolic reprogramming of MDSCs and influence their differentiation and maintenance. Compounds that target ROS in MDSCs to enhance the effects of cancer immune therapy are promising therapeutic options. The synthetic triterpenoid C-28 methyl ester of 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid (CDDO-Me, also referred to as bardoxolone methyl, RTA402, TP-155 and NSC713200) is a potent Nrf2 activator and has been found to reduce MDSC production of ROS and tumor growth in mouse tumor models (62). CDDO-Me shows a promising anticancer effect in a phase I trial (63). In addition, systemic treatment with all-trans-retinoic acid (ATRA) promotes maturation of human MDSCs and reverses their immune suppressor function. Accumulation of GSH in MDSCs by ATRA decreases levels of ROS and induces MDSC differentiation into mature myeloid cells (30, 64). Until now, most studies have focused on cancer models and suggest that inhibition of ROS production in MDSCs helps to enhance anti-tumor immune responses. Beyond their pathogenic role in cancer, expansion and activation of MDSCs also occurs in autoimmunity, infection and chronic inflammation, conditions that are associated with oxidative stress and hypoxic states (10, 65, 66). Thus, redox-signaling in MDSCs might be a promising therapeutic target in these diseases as well. However, the role of MDSCs here seems to be less clear here, and both positive and negative roles of MDSCs have been revealed with regard to progression of autoimmune diseases. Therefore, further studies are warranted to uncover the specific role of redox signaling in MDSCs in autoimmunity and infection.

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REFERENCES

1. Kalyanaraman B, Cheng G, Hardy M, Ouari O, Bennett B, Zielonka J. Teaching the basics of reactive oxygen species and their relevance to cancer biology: Mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies. *Redox Biol.* (2018) 15:347–62. doi: 10.1016/j.redox.2017.12.012
2. Sareila O, Kelkka T, Pizzolla A, Hultqvist M, Holmdahl R. NOX2 complex-derived ROS as immune regulators. *Antioxid Redox Signal* (2011) 15:2197–208. doi: 10.1089/ars.2010.3635
3. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* (2009) 9:162–74. doi: 10.1038/nri2506
4. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol.* (2009) 182:5693–701. doi: 10.4049/jimmunol.0900092
5. Manjili MH, Wang XY, Abrams S. Evolution of our understanding of myeloid regulatory cells: from MDSCs to Mregs. *Front Immunol.* (2014) 5:303. doi: 10.3389/fimmu.2014.00303
6. Beury DW, Carter KA, Nelson C, Sinha P, Hanson E, Nyandjo M, et al. Myeloid-derived suppressor cell survival and function are regulated by the transcription factor Nrf2. *J Immunol.* (2016) 196:3470–8. doi: 10.4049/jimmunol.150178
7. Dietlin TA, Hofman FM, Lund BT, Gilmore W, Stohlman SA, van der Veen RC. Mycobacteria-induced Gr-1+ subsets from distinct myeloid lineages have opposite effects on T cell expansion. *J Leukoc Biol.* (2007) 81:1205–12. doi: 10.1189/jlb.1006640
8. Ohl K, Tenbrock K, Kipp M. Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp Neurol.* (2016) 277:58–67. doi: 10.1016/j.expneurol.2015.11.010
9. Kaminski MM, Roth D, Krammer PH, Gulow K. Mitochondria as oxidative signaling organelles in T-cell activation: physiological role and pathological implications. *Arch Immunol Ther Exp.* (2013) 61:367–84. doi: 10.1007/s00005-013-0235-0
10. Novak EA, Mollen KP. Mitochondrial dysfunction in inflammatory bowel disease. *Front Cell Dev Biol.* (2015) 3:62. doi: 10.3389/fcell.2015.00062
11. Chen Y, Zhang H, Zhou HJ, Ji W, Min W. Mitochondrial redox signaling and tumor progression. *Cancers* (2016) 8:40. doi: 10.3390/cancers8040040
12. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol.* (2013) 13:349–61. doi: 10.1038/nri3423

13. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol*. (2003) 23:8137–51. doi: 10.1128/MCB.23.22.8137-8151.2003
14. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol*. (2007) 47:89–116. doi: 10.1146/annurev.pharmtox.46.120604.141046
15. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cell Mol Immunol*. (2015) 12:5–23. doi: 10.1038/cmi.2014.89
16. Quie PG, White JG, Holmes B, Good RA. *In vitro* bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. *J Clin Invest*. (1967) 46:668–79. doi: 10.1172/JCI105568
17. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* (2011) 472:476–80. doi: 10.1038/nature09973
18. Gurung P, Lukens JR, Kanneganti TD. Mitochondria: diversity in the regulation of the NLRP3 inflammasome. *Trends Mol Med*. (2015) 21:193–201. doi: 10.1016/j.molmed.2014.11.008
19. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* (2011) 469:221–5. doi: 10.1038/nature09663
20. Dan Dunn J, Alvarez LA, Zhang X, Soldati T. Reactive oxygen species and mitochondria: a nexus of cellular homeostasis. *Redox Biol*. (2015) 6:472–85. doi: 10.1016/j.redox.2015.09.005
21. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med*. (2010) 207:2439–53. doi: 10.1084/jem.20100587
22. Ohl K, Fragoulis A, Klemm P, Baumeister J, Klock W, Verjans E, et al. Nrf2 Is a central regulator of metabolic reprogramming of myeloid-derived suppressor cells in steady state and sepsis. *Front Immunol*. (2018) 9:1552. doi: 10.3389/fimmu.2018.01552
23. Jian SL, Chen WW, Su YC, Su YW, Chuang TH, Hsu SC, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. *Cell Death Dis*. (2017) 8:e2779. doi: 10.1038/cddis.2017.192
24. Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* (2014) 345:1250684. doi: 10.1126/science.1250684
25. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol*. (2016) 16:553–65. doi: 10.1038/nri.2016.70
26. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *Cancer Immunol Res*. (2015) 3:1236–47. doi: 10.1158/2326-6066.CIR-15-0036
27. Wu T, Zhao Y, Wang H, Li Y, Shao L, Wang R, et al. mTOR masters monocytic myeloid-derived suppressor cells in mice with allografts or tumors. *Sci Rep*. (2016) 6:20250. doi: 10.1038/srep20250
28. Holmstrom KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol*. (2014) 15:411–21. doi: 10.1038/nrm3801
29. Mak TW, Grusdat M, Duncan GS, Dostert C, Nonnenmacher Y, Cox M, et al. Glutathione primes T cell metabolism for inflammation. *Immunity* (2017) 46:675–89. doi: 10.1016/j.immuni.2017.03.019
30. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res*. (2007) 67:11021–8. doi: 10.1158/0008-5472.CAN-07-2593
31. Kusmartsev S, Gabrilovich DI. Inhibition of myeloid cell differentiation in cancer: the role of reactive oxygen species. *J Leukoc Biol*. (2003) 74:186–96. doi: 10.1189/jlb.0103010
32. Chen X, Song M, Zhang B, Zhang Y. Reactive oxygen species regulate T cell immune response in the tumor microenvironment. *Oxid Med Cell Longev*. (2016) 2016:1580967. doi: 10.1155/2016/1580967
33. Zhang H, Li ZL, Ye SB, Ouyang LY, Chen YS, He J, et al. Myeloid-derived suppressor cells inhibit T cell proliferation in human extranodal NK/T cell lymphoma: a novel prognostic indicator. *Cancer Immunol Immunother*. (2015) 64:1587–99. doi: 10.1007/s00262-015-1765-6
34. Wei J, Zhang M, Zhou J. Myeloid-derived suppressor cells in major depression patients suppress T-cell responses through the production of reactive oxygen species. *Psychiatry Res*. (2015) 228:695–701. doi: 10.1016/j.psychres.2015.06.002
35. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med*. (2007) 13:828–35. doi: 10.1038/nm1609
36. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res*. (2001) 61:4756–60.
37. Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res*. (2006) 66:1123–31. doi: 10.1158/0008-5472.CAN-05-1299
38. Garcia MR, Ledgerwood L, Yang Y, Xu J, Lal G, Burrell B, et al. Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice. *J Clin Invest*. (2010) 120:2486–96. doi: 10.1172/JCI41628
39. Park MJ, Lee SH, Kim EK, Lee EJ, Baek JA, Park SH, et al. Interleukin-10 produced by myeloid-derived suppressor cells is critical for the induction of Tregs and attenuation of rheumatoid inflammation in mice. *Sci Rep*. (2018) 8:3753. doi: 10.1038/s41598-018-21856-2
40. Nagaraj S, Youn JI, Gabrilovich DI. Reciprocal relationship between myeloid-derived suppressor cells and T cells. *J Immunol*. (2013) 191:17–23. doi: 10.4049/jimmunol.1300654
41. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol*. (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
42. Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol*. (2011) 11:802–7. doi: 10.1016/j.intimp.2011.01.003
43. Kraaij MD, Savage ND, van der Kooij SW, Koekkoek K, Wang J, van den Berg JM, et al. Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species. *Proc Natl Acad Sci USA*. (2010) 107:17686–91. doi: 10.1073/pnas.1012016107
44. Mougiakakos D, Johansson CC, Kiessling R. Naturally occurring regulatory T cells show reduced sensitivity toward oxidative stress-induced cell death. *Blood* (2009) 113:3542–5. doi: 10.1182/blood-2008-09-181040
45. Pae HO, Oh GS, Choi BM, Chae SC, Chung HT. Differential expressions of heme oxygenase-1 gene in CD25- and CD25+ subsets of human CD4+ T cells. *Biochem Biophys Res Commun*. (2003) 306:701–5. doi: 10.1016/S0006-291X(03)01037-4
46. Maj T, Wang W, Crespo J, Zhang H, Wang W, Wei S, et al. Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor. *Nat Immunol*. (2017) 18:1332–41. doi: 10.1038/ni.3868
47. Lu T, Ramakrishnan R, Altiok S, Youn JI, Cheng P, Celis E, et al. Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. *J Clin Invest*. (2011) 121:4015–29. doi: 10.1172/JCI45862
48. Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med*. (2011) 208:1949–62. doi: 10.1084/jem.20101956
49. Fortin C, Huang X, Yang Y. NK cell response to vaccinia virus is regulated by myeloid-derived suppressor cells. *J Immunol*. (2012) 189:1843–9. doi: 10.4049/jimmunol.1200584
50. Zhu J, Huang X, Yang Y. Myeloid-derived suppressor cells regulate natural killer cell response to adenovirus-mediated gene transfer. *J Virol*. (2012) 86:13689–96. doi: 10.1128/JVI.01595-12
51. Liu C, Yu S, Kappes J, Wang J, Grizzle WE, Zinn KR, et al. Expansion of spleen myeloid suppressor cells represses NK cell cytotoxicity in tumor-bearing host. *Blood* (2007) 109:4336–42. doi: 10.1182/blood-2006-09-046201
52. Rastad JL, Green WR. Myeloid-derived suppressor cells in murine AIDS inhibit B-cell responses in part via soluble mediators including reactive oxygen and nitrogen species, and TGF-beta. *Virology* (2016) 499:9–22. doi: 10.1016/j.virol.2016.08.031

53. Green KA, Cook WJ, Green WR. Myeloid-derived suppressor cells in murine retrovirus-induced AIDS inhibit T- and B-cell responses *in vitro* that are used to define the immunodeficiency. *J Virol.* (2013) 87:2058–71. doi: 10.1128/JVI.01547-12
54. Lelis FJN, Jaufmann J, Singh A, Fromm K, Teschner AC, Poschel S, et al. Myeloid-derived suppressor cells modulate B-cell responses. *Immunol Lett.* (2017) 188:108–15. doi: 10.1016/j.imlet.2017.07.003
55. Chiu DK, Tse AP, Xu IM, Di Cui J, Lai RK, Li LL, et al. Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma. *Nat Commun.* (2017) 8:517. doi: 10.1038/s41467-017-00530-7
56. Liu G, Bi Y, Shen B, Yang H, Zhang Y, Wang X, et al. SIRT1 limits the function and fate of myeloid-derived suppressor cells in tumors by orchestrating HIF-1alpha-dependent glycolysis. *Cancer Res.* (2014) 74:727–37. doi: 10.1158/0008-5472.CAN-13-2584
57. Li J, Wang L, Chen X, Li L, Li Y, Ping Y, et al. CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-beta-mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncoimmunology* (2017) 6:e1320011. doi: 10.1080/2162402X.2017.1320011
58. Noman MZ, Janji B, Hu S, Wu JC, Martelli F, Bronte V, et al. Tumor-promoting effects of myeloid-derived suppressor cells are potentiated by hypoxia-induced expression of miR-210. *Cancer Res.* (2015) 75:3771–87. doi: 10.1158/0008-5472.CAN-15-0405
59. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med.* (2014) 211:781–90. doi: 10.1084/jem.20131916
60. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, Suzuki T, et al. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. *Carcinogenesis* (2010) 31:1833–43. doi: 10.1093/carcin/bgq105
61. Moon EJ, Giaccia A. Dual roles of NRF2 in tumor prevention and progression: possible implications in cancer treatment. *Free Rad Biol Med.* (2015) 79:292–9. doi: 10.1016/j.freeradbiomed.2014.11.009
62. Nagaraj S, Youn JI, Weber H, Iclozan C, Lu L, Cotter MJ, et al. Anti-inflammatory triterpenoid blocks immune suppressive function of MDSCs and improves immune response in cancer. *Clin Cancer Res.* (2010) 16:1812–23. doi: 10.1158/1078-0432.CCR-09-3272
63. Wang YY, Yang YX, Zhe H, He ZX, Zhou SF. Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. *Drug Des Dev Therapy* (2014) 8:2075–88. doi: 10.2147/DDDT.S68872
64. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, et al. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res.* (2006) 66:9299–307. doi: 10.1158/0008-5472.CAN-06-1690
65. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. *PloS ONE* (2016) 11:e0152925. doi: 10.1371/journal.pone.0152925
66. Brik R, Livnat G, Pollack S, Catz R, Nagler R. Salivary gland involvement and oxidative stress in juvenile idiopathic arthritis: novel observation in oligoarticular-type patients. *J Rheumatol.* (2006) 33:2532–7.

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REVIEW
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MDSCs: Key Criminals of Tumor Pre-metastatic Niche Formation

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The emergence of disseminated metastases remains the primary cause of mortality in cancer patients. Formation of the pre-metastatic niche (PMN), which precedes the establishment of tumor lesions, is critical for metastases. Bone marrow-derived myeloid cells (BMDCs) are indispensable for PMN formation. Myeloid-derived suppressor cells (MDSCs) are a population of immature myeloid cells that accumulate in patients with cancer and appear in the early PMN. The mechanisms by which MDSCs establish the pre-metastatic microenvironment in distant organs are largely unknown, although MDSCs play an essential role in metastasis. Here, we summarize the key factors associated with the recruitment and activation of MDSCs in the PMN and review the mechanisms by which MDSCs regulate PMN formation and evolution. Finally, we predict the potential value of MDSCs in PMN detection and therapy.

Keywords: metastasis, pre-metastatic niche, myeloid-derived suppressor cells, formation, evolution, detection

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INTRODUCTION

Metastasis remains the leading cause of cancer-related death. Decades of investigations into cancer metastasis have focused largely on the causes of oncogenic transformation and the incipient emergence of tumors, although Stephen Paget proposed the seed-and-soil hypothesis in 1889 (1). Metastasis-related high mortality has driven cancer biologists to renew their focus on the problem of metastasis. The study of how tumor cells lead to metastasis, such as altering the microenvironment, entering the circulation, and colonizing distant organs, has received more attention. Tireless research efforts have revealed that metastasis results from the interplay of wandering tumor cells with a supportive microenvironment in target tissues (2). The theory that a preconditioned microenvironment that receives incoming cancer cells at secondary organs or sites, termed the PMN, is the key determinant of cancer metastasis is widely accepted. Fidler et al. found that although mouse B16 melanoma cells could be found in the vasculature of multiple organs (3), only lung sites consistently developed metastatic tumor deposits, which provided support for this theory. Kaplan's research in 2005 first demonstrated the existence and stepwise progression of the PMN in Lewis lung carcinoma cells (LLC) or B16 cell-bearing mice (4). However, the complex processes and molecular mechanisms involved in PMN formation have remained among the greatest mysteries surrounding cancer metastasis.

This supportive PMN is prepared by resident cells (5), recruited bone marrow-derived cells (BMDCs) (4), soluble factors (6), and extracellular vesicles (EVs) (7, 8). BMDCs are the main cellular components of the PMN, which is initiated by many types of primary tumors, including colorectal cancer (9, 10), breast cancer (11), and melanoma (12). The evidence of BMDCs in

PMN formation is primarily drawn from mouse models and largely focused on the lung and liver as a target organ, although other organs and pathological samples from patients have also been examined. Lewis lung carcinoma (LLC) cells and B16 melanoma cells possess a more widely disseminated metastatic potential and tend to metastasize to the lungs and liver. LLC or B16 tumors are more general models for the PMN related research. Rosandra et al. confirmed the role of BMDCs in PMN formation through LLC or B16 tumors (4). In this study, C57BL/6 mice were lethally irradiated and transplanted with GFP^+ bone marrow cells. Mice were injected intradermally with either LLC or B16 cells. After irradiation, but before tumor implantation, minimal BMDCs were observed in the lungs or liver. After tumor implantation, but before the arrival of tumor cells, the extravasation and cluster formation of BMDCs were detected near distal alveoli and terminal bronchioles, both common sites for future tumor metastasis. Until day 16, tumor cells were detected and more than 95% of tumor cells co-clustered with GFP^+ BMDCs. Therefore, factors provided by the primary tumor promote BMDCs to mobilize to pre-metastatic sites, and this migration precedes the arrival of tumor cells. However, the mechanisms by which BMDCs mediate the outgrowth of metastatic cancer cells are not completely understood.

Neutrophils can be expanded, mobilized and recruited to the PMN when the primary tumor occurs. However, the role of neutrophils in PMN formation is not consistent. In colorectal cancer model mice, tissue inhibitor of metalloproteinases (TIMP)-1 creates a PMN in the liver through SDF-1/CXCR4-dependent neutrophil recruitment (13). In mouse models of breast cancer, G-CSF-mobilized $Ly6G^+Ly6C^+$ granulocytes home to distant organs before the arrival of tumor cells and produce the Bv8 protein, which stimulates tumor cell migration through activation of prokineticin receptor (PKR)-1 (14). This result is also observed during early breast cancer progression, G-CSF directs the production of T cell-suppressive neutrophils, which preferentially accumulate in peripheral tissues but not in the primary tumor (15). Specifically, tumor-secreted CCL2 stimulates neutrophils to accumulate in the lung prior to the arrival of metastatic cells and inhibits metastatic seeding by generating H_2O_2 in breast cancer mice (16). Thus, neutrophils could promote PMN formation. However, another study in breast cancer mice showed that neutrophils kill tumor cells through ROS production and granzyme-B release (17). Therefore, neutrophils can be a double-edged sword in PMN formation. Currently, the markers used to define neutrophils are oversimplified, and these neutrophils cannot actually be distinguished from MDSCs. Therefore, phenotypic analysis of these neutrophils is still a matter of study and deeper immunophenotyping and functional assessment of PMN-infiltrating immune cells are required.

MDSCs are a heterogeneous group of myeloid cells with immunosuppressive properties that are derived from myeloid progenitor cells and immature myeloid cells. MDSCs have been detected in the lungs of mice bearing mammary adenocarcinoma prior to metastatic spread (18). MDSCs have been shown to play pleiotropic roles in cancer progression by shaping the tumor microenvironment and metastatic niches through immunosuppression and inflammation. Expanding experimental evidence indicates that MDSCs are the key determinants of PMN formation, although other immune cells, such as neutrophil, macrophage and Tregs also involved in PMN formation (19). S100A8/A9 imaging shows that MDSCs are abundant in the pre-metastatic lung and correlate with the subsequent metastatic breast cancer burden (20). In breast cancer model mice, MDSCs accumulate in the PMN and suppress cytotoxic $CD8^+$ T cells and NK cells through the productions of reactive oxygen species (ROS) and arginase 1 (Arg-1) (21). MDSCs are also involved in an array of non-immunological functions that may be associated with the PMN through secretion of cytokines, chemokine's, growth factors and exosomes. The roles of MDSCs in PMN formation and evolution are diverse and may range from the induction of vascular leakage and extracellular matrix (ECM) remodeling to systemic effects on the immune system that facilitate metastatic outgrowth (19, 22, 23).

In this review, we summarize the new phenotypic features of MDSCs and the main factors that regulate MDSC recruitment and expansion in the PMN. We mainly discuss the multi-faceted superior capacity of MDSCs to establish a pre-metastatic microenvironment in distant organs, and finally provide new insights into how this process can be translated into clinical applications.

MDSC PHENOTYPE AND FUNCTION

MDSCs are a heterogeneous population of immature myeloid cells whose numbers are increased in states of cancer, inflammation, or infection (24, 25). At present, most knowledge about MDSCs comes from tumor immunity research. Tumor cells mobilize MDSC differentiation, proliferation, and migration toward tumor tissue by secreting vascular endothelial growth factor (VEGF), granulocyte-macrophage colony stimulating factor (GM-CSF), IL-6, IL-10, transforming growth factor-β (TGF-β) and other factors (26). The heterogeneity of MDSCs is derived from the complex expression patterns of their surface markers and locations. MDSCs mainly include monocytic MDSC (M-MDSC) and granulocytic MDSC (G-MDSC) (or polymorphonuclear MDSC, PMN-MDSC) subpopulations. Other subsets further characterized in human MDSC are the immature MDSC [also known "early-stage MDSC" [eMDSC]] and fibrocytic MDSCs (F-MDSCs). MDSC subsets have been characterized (27), and play critical roles in tumor progression through different mechanisms (**Table 1**) (32–37). It is worth noting that the dynamic interplay between cancer and host immune system often affects the process of myelopoiesis. The difference of MDSCs locations have contributed to the complex expression patterns of surface markers and effector molecules (**Table 1**) (28–30). G-MDSCs and M-MDSCs use different mechanisms for immunosuppression, which have been reviewed elsewhere (31, 39, 40). Briefly, G-MDSCs mainly suppress T cell responses by producing ROS (reactive oxygen species) via an antigen-specific approach. M-MDSCs produce high amounts of NO, Arg-1 and immunosuppressive cytokines, such as IL-10, which suppress both antigen-specific and non-specific T cell

responses. M-MDSCs have higher suppressive activity than G-MDSCs. F-MDSCs suppress T cell proliferation through IDO (indoleamine oxidase) production and promote Treg cell expansion.

MDSCs are defined based on their phenotypic, functional, and molecular features. Notably, the expression levels of the molecules often change with environmental changes. Primary tumor derived factors mobilize MDSCs from bloodstream into the tissues where metastasis is about to occur. In pre-metastatic lung tissue, MDSCs are indicative of the granulocytic nature of $CD11b^{+}Ly6C^{lo/med}Ly6G^{+}$ cells which is referred to as G-MDSC or PMN-MDSC. Compared to neutrophils, these cells have fewer granules, diminished CD62L, and CD16 expression. Moreover, these cells express a high level of ROS and Arg-1(31). In mice bearing mammary adenocarcinoma, MDSCs have been detected in the pre-metastatic lung. Phenotypic analysis revealed that $Ly6G^{+}Ly6C^{lo}$ cells constituted the major share of such cells, which was indicative of G-MDSCs (18). MDSCs in pre-metastatic tissue provide an microenvironment that is suitable for the arrival and settlement of tumor cells through promoting immunosuppression, leaky vasculature, and collagen restructuring in the premetastatic tissue.

Researchers also define MDSC subpopulations using intrinsic and extrinsic cell death pathway properties, which are involved in myeloid lineage development and survival. The anti-apoptotic molecule cellular Fas-associated death domain-like interleukin-1 β converting enzyme inhibitory protein (c-FLIP) is constitutively required for the development of M-MDSCs, whereas G-MDSCs require a different anti-apoptotic molecule [myeloid cell leukemia 1 (MCL-1)] for development (41). The ability to suppress immune cells is an important standard that is used to define MDSCs. Suppression of T cell activity, including reduced proliferation and suppressed IFN-γ and IL-2 production, is an important standard for the evaluation of MDSC immunosuppressive function (33). The phenotypic and functional characteristics of MDSCs in the PMN need to be further investigated, although current studies have shown that MDSCs in the PMN originate from the bone marrow. In addition, the role of the PMN in MDSC functions and phenotypes is unclear.

PMN FORMATION AND EVOLUTION

Previous studies investigating tumor metastasis focused largely on identifying cancer cell intrinsic determinants, such as genes and pathways that regulate colonization. Currently, promotion of the spread of tumor cells to secondary organs by prior formation of a supportive PMN at distant sites before the arrival of metastatic cells is widely accepted. The PMN has become a new paradigm for the initiation of metastasis, although understanding the complexity of the PMN is daunting. Indeed, a number of elements are involved in the formation and evolution of the PMN, including cells from different lineages, blood flow, soluble factors, EVs, extracellular matrix, and signaling molecules that can provide niches for tumor settlement and growth. The pathological processes that occur before the development of macrometastases require better understanding. Kaplan's research in 2005 first demonstrated the existence and stepwise progression of the PMN (4). Researchers have uncovered the usual progression of PMN formation in diverse tumors, including colorectal cancer (9, 10), breast cancer (11) and melanoma (12, 42). First, tumor-secreted factors, the effects of surgery, infection and aging not only change blood flow and vascular leakage but also contribute to activation and recruitment of BMDC populations (43, 44). Second, the biological behavior of resident cells changes, and the ECM in the PMN is remodeled (7, 42, 45). Third, a microenvironment with inflammation, immunosuppression, and coagulation disorders is established, which is beneficial for the ability of arriving tumor cells to settle down and survive (19).

REGULATION OF MDSC RECRUITMENT AND ACTIVATION IN THE PMN

Significant advancements have been made in understanding the regulation of MDSC accumulation and expansion in primary tumors. Currently, diverse factors, including GM-CSF (46), interleukins (47),VEGF (48), tumor-derived molecules (49), prostaglandin E2/cyclooxygenase-2 (PGE2/COX2) (50), EVs (51), complement molecules (52), and IFN-γ (53), have been determined to regulate MDSC accumulation and expansion in the tumor microenvironment through the signal transducers and activators of transcription 1 (STAT1) or STAT3 signaling pathway (54). However, how MDSCs migrate into pre-metastatic sites and become activated is unclear, although research results have shown that MDSCs can infiltrate into the PMN in the presence of soluble factors, including GM-CSF, VEGF, IL-6, IL-1β, and CCL2 (55). The main factors that affect the accumulation and activation of MDSCs in PMN are summarized in **Table 2**.

Chemokines

Chemokines and other soluble factors secreted by tumors and stromal cells are the main components that affect the migration and activation of MDSCs in the PMN. Primary tumor cells and stromal cells secreted factors and EVs drive the expansion of MDSCs within the bone marrow and enhance actin polymerization in MDSCs and vascular leakiness in the bone marrow (BM) and PMN, which create conditions conducive for the mobilization of MDSC from BM to secondary sites (**Figure 1**). In colorectal cancer, VEGF secretion by colorectal carcinoma cells stimulates tumor-associated macrophages (TAMs) to produce chemokine (C-X-C motif) ligand 1 (CXCL1), which recruits C-X-C motif chemokine receptor 2 $(CXCR2)^{+}$ MDSCs to the liver tissue. The accumulated MDSCs promote PMN formation and ultimately promote liver metastases (56). CCL2 is also referred to as monocyte chemoattractant protein 1 (MCP1), was demonstrated to be a functional contributor to PMNs. In a murine liver tumor model, tumor-associated fibroblast-secreted CCL2 induces mobilization and migration of MDSCs to the PMN through chemokine receptor 2 (CCR2) (66). In a mouse breast cancer lung metastasis animal model, CCL2 also promotes

TABLE 1 | Phenotype and function of MDSCs.

Subset	Phenotype (Mouse)	Phenotype (Human)
Total MDSC	$CD11b^{+}Gr\text{-}1^{+}CD11c^{-}F4/80^{+/-}CD124^{+}$	$HLA\text{-}DR^{-}CD11b^{+}CD33^{+}$
G-MDSC	$CD11b^{+}Gr\text{-}1^{hi}Ly6C^{low}Ly6G^{+}CD49d^{-}$	$CD33^{+}CD14^{-}CD11b^{+}CD15^{+}$(or $CD66b^{+}$)
M-MDSC	$CD11b^{+}Gr\text{-}1^{mid}Ly6C^{hi}Ly6G^{-}CD49d^{+}$	$CD11b^{+}CD14^{+}HLA\text{-}DR^{low/-}CD15^{-}$
e-MDSC	–	Lin^{-}(CD3/14/15/19/56) $HLA\text{-}DR^{-}CD33^{+}$
F-MDSC	–	$CD11b^{low}CD11c^{low}CD33^{+}$ $IL\text{-}4Ra^{+}$
Category	**Surface molecule (Mouse)**	**Effector molecule (Mouse)**
BM derived progenitors	CD133, CD34, CD117, VLA-4	ROS (28)
MDSCs in BM	CD11b, Ly6G, Ly6C	ROS, $Bv8^{low}$ (29)
MDSCs in blood	CD11b, Ly6G, Ly6C	ROS, Bv8 (29, 30)
Tumor-infiltrating MDSCs	CD11b, Ly6G, Ly6C, CD115, F4/80, CD80	Arg-1, iNOS, $NO2^{-}$, Bv8 (29, 30)
MDSCs in PMN	CD11b, Ly6G, $Ly6C^{low}$, $CD62L^{low}$, $CD16^{low}$	ROS, Arg-1 (18, 31)

Function	Description
Immune suppression	Inhibit T-cell proliferation, NK cell and CTL activity, IL-2 production, and promote Treg induction and M2 macrophage reprogramming through secreting Arg-1, ROS, NOS2, IDO, TGF-β, IL-10, and exosomes or membrane molecules (32, 33).
Tumor angiogenesis	Promote blood vessel formation through upregulating MMP9, VEGF, and Bv8 expression (34, 35).
Tumor cell stemness	Trigger miR-101 expression and target the CtBP2 (36).
Metastasis dissemination	Support the epithelial-mesenchymal transition through secreting hepatocyte growth factor and TGF-β1 (37). Regulate resident cell and angiogenesis through exosomal miRNA (38).

TABLE 2 | Factors associated with MDSC accumulation/activation in the PMN.

Molecules	Source	Receptors	Phenotype	Model	Sites	References
CXCL1	TAMs	CXCR2	$CXCR2^{+}$ MDSCs	Colorectal carcinoma	Liver	(49)
CCL12	Lung	–	M-MDSCs	Melanoma	Lung	(52)
MCP-1/CCL2	BMDCs	CCR2	MDSCs	Skin/Breast cancer	Skin/Lung	(21, 56)
CXCL12	HSCs	CXCR4	MDSCs	Pancreatic tumor	Liver	(57)
CCL15	Colorectal tumor cells	CCR1	$CCR1^{+}$ MDSCs	Colorectal cancer	Liver	(55)
CCL9	G-MDSCs	CCR1	G-MDSCs	Melanoma/Breast cancer	Lung	(54)
Exosomal Hsp72	Tumor cells	TLR2	MDSCs	Colon carcinoma	–	(58)
Exosomal MET	Melanoma	–	MDSCs	Melanoma	Lung	(12)
S100A8/9	MDSCs	TLR4	MDSCs	Breast/ Gastric/ Lung cancer	Lung	(59)
Periostin	MDSCs	–	M/G-MDSCs	Breast tumor	Lung	(60)
ER stress	Neutrophils	–	$LOX\text{-}1^{+}$ PMN-MDSCs	HNC NSCLC	Lung	(61)
LOX	Breast tumor cell	–	$CD11b^{+}$ myeloid cells	Breast tumor	Lung	(62)
G-CSF	–	–	MDSC	Melanoma/Lung cancer/Lymphoma	–	(63)
FN	Fibroblasts	VLA-4	$VEGFR1^{+}$ HPCs	Lung cancer	Lung	(4)
VEGF	Ovarian tumor cells	VEGFR1	MDSCs	Ovarian cancer	PN	(47)
TGF-β	Melanoma cells		$Id1^{high}$MDSCs	Melanoma	–	(64)
SAA	ECs	TLR4	$CD11b^{+}$ myeloid cells	Lung cancer	Lung	(65)
miRNA9	MDSCs	–	MDSCs	Lung cancer	–	(24)

MDSC migration and triggers S100A8/A9 secretion (20). In breast cancer, monocyte chemoattractant protein 1 (MCP-1) recruits PMN-MDSCs to the pre-metastatic lung and suppresses NK cell function, which promotes the formation of an immunosuppressive PMN (21).BMDCs express CCL2 to attract MDSCs via CCR2 in hedgehog-induced skin tumors (67). Furthermore, CCL12 promotes M-MDSCs to migrate to premetastatic lungs in melanoma cell-bearing mice and increases IL-1β and E-selectin expression before the arrival of tumor cells, which is beneficial for tumor cell arrest of endothelial cells (68). In addition, CCL9 is an important factor supporting MDSC recruitment to future PMNs. In colorectal cancer, CCL9 from the tumor epithelium recruits immature myeloid cells via the CCR1 receptor, which promotes tumor

invasion (69). In melanoma and breast cancer-bearing mice, TGF-β regulates CCL9 production in MDSCs through p38, which shows a CCL9-CCR1 autocrine effect on MDSC survival through decreasing cell apoptosis (70). Moreover, CCL9 increases the levels of phosphorylated protein kinase B (p-PKB) and B-cell lymphoma-2 (Bcl-2) in tumor cells, which promote the survival of newly arriving tumor cells in the PMN (70). Last but not least, serum CCL15 also promotes MDSC recruitment through CCR1, which is beneficial for colorectal cancer cell metastasis to the liver (71). It is worth noting that primary tumor also enhance BM progenitors mobilization to the PMN through factors or exosomes secretion and these progenitors may further differentiate into MDSCs. Peinado et al. confirmed that melanoma exosomes reprogrammed BM progenitors toward a c-Kit$^+$Tie2$^+$Met$^+$ pro-vasculogenic phenotype and enhanced these progenitors mobilization to the prometastatic lung through MET (7). In mouse models of metastatic lung, during the angiogenic switch, bone marrow-derived hematopoietic progenitor cells expressing VEGFR1 proliferate and mobilize to the bloodstream. These cells home to LLC cells -specific pre-metastatic lungs and form cellular clusters before the arrival of tumor cells, which metastasize to the lungs (72). Further characterization of cellular clusters revealed that these cells expressed myelomonocytic marker CD11b and secreted MMP9 (72). Overall, the role of these chemokines in the formation and evolution of PMN must be taken. Such chemokines may be the targets to block the formation of PMN.

Integrins

Integrins are transmembrane receptors that facilitate cell-extracellular matrix adhesion. The very late antigen-4 (VLA-4) integrin is expressed by numerous cells of haematopoietic origin and possesses a key function in the cellular immune response and cancer metastasis (73). Bone marrow-derived VEGFR1$^+$VLA-4$^+$ HPCs migrate to the PMN and interact with resident fibroblasts through the fibronectin ligand of VLA-4, resulting in the formation of cellular clusters. These expression patterns of fibronectin and VEGFR1$^+$VLA-4$^+$ clusters foster a supportive microenvironment for incoming LLC or melanoma B12 cells and dictate organ-specific tumor spread (4). Blocking VLA-4 reduces tissue infiltration of M-MDSCs through inhibiting adherence to the apical side of the endothelium during the pathogenic process underlying hepatic inflammation (74). These results suggest that VLA-4 is a key molecule that regulates MDSC infiltration into tissues and may serve as an important target for blocking PMN formation. Future studies should focus on exploring strategies for blocking PMN formation based VLA-4.

ECM Remodeling-Related Factors

The ECM remodeling-related factors contributes to many aspects of tumor progression by acting on both tumor and immune cells. In particular, ECM remodeling-related factors-mediated regulation of immunosuppression occurs through regulation of the expansion, localization, and functional activities of myeloid cells (75). The calcium binding protein S100A8/A9 is a damage-associated molecular pattern which can activate Toll-like receptor (TLR)-4 or receptor for advanced glycation end-products (RAGE). Activation of these receptors is involved in the recruitment of MDSCs. In LLC-bearing mice, S100A8 promotes MDSC recruitment through p38 and NF-κB activation in a TLR4-dependent manner (59). In mammary carcinoma cell-bearing mice, S100A8/A9 from myeloid and tumor cells bind to RAGE on MDSCs and promote MDSC migration and accumulation through the NF-κB signaling pathways (76, 77). Periostin, which is a non-structural ECM protein, is a limiting factor in the metastatic colonization of disseminated tumor cells. Periostin promotes the pulmonary accumulation of MDSCs during the early stage of breast tumor metastasis (60). In periostin-deficient MDSCs, the activation of extracellular regulated protein kinase (ERK), PKB and STAT3 and immunosuppressive functions are decreased, which accelerate breast tumor growth (60). These results indicate that periostin from MDSCs participates in PMN formation through promoting ECM remodeling and regulates the activation and function of MDSCs. In addition, periostin also elevate Lysyl oxidase (LOX) activity. LOX is an extracellular matrix, copper-dependent amine oxidase that catalyzes a key enzymatic step in the crosslinking of collagen (78). In PMN, LOX promotes the crosslinking of collagen IV in the basement membrane. Cross-linked collagen IV is essential for CD11b$^+$ myeloid cell recruitment (60). CD11b$^+$ cells adhere to cross-linked collagen IV and produce matrix metalloproteinase-2 (MMP2). MMP2 cleaves collagen, enhancing the invasion and recruitment of bone marrow-derived cells (62), which promote PMN formation. PMN-MDSCs are important regulators of immune responses in cancer and have been directly implicated in promotion of PMN formation. Lectin-type oxidized LDL receptor-1 (LOX-1) is a distinct surface marker for human PMN-MDSC. Endoplasmic reticulum (ER) stress converts neutrophils from healthy donors to suppressive G-MDSCs through increasing LOX-1 expression (61). In patients with hepatocellular carcinoma, ER stress promote LOX-1$^+$CD15$^+$ G-MDSCs expansion and suppress T cell proliferation through ROS/Arg-1(79). These results suggest that significant ER stress in a tumor-bearing host might induce PMN formation mediated by enhancement of LOX-1$^+$CD15$^+$ G-MDSCs -mediated suppression. In tumor-bearing mice transplanted with B16F1, Tib6, EL4, or LLC cells, tumor-secreted granulocyte colony-stimulating factor (G-CSF) mobilizes peripheral CD11b$^+$Gr1$^+$ cells to the pre-metastatic lung (63). VEGFA from ovarian cancer cells promotes MDSC migration and differentiation through VEGFR1, which is expressed on MDSCs, and suppress CD8$^+$ T cell infiltration (48). In melanoma tumors, TGF-β mediated inhibitor of differentiation 1 (Id1) upregulation skews dendritic cell differentiation to MDSCs and mobilizes VEGFR1$^+$ haematopoietic progenitor cells (HPCs) during PMN formation (64). In addition, serum amyloid A(SAA) 3, an acute phase protein, stimulates proliferative, and proinflammatory responses of keratinocytes, also participate in the formation of PMN. In LLC or B16 cell-bearing mice, serum amyloid A (SAA) 3 from endothelial cells and alveolar macrophages also attracts CD11b$^+$ myeloid cells into pre-metastatic lungs (65). Therefore, extracellular matrix proteins play a major role in

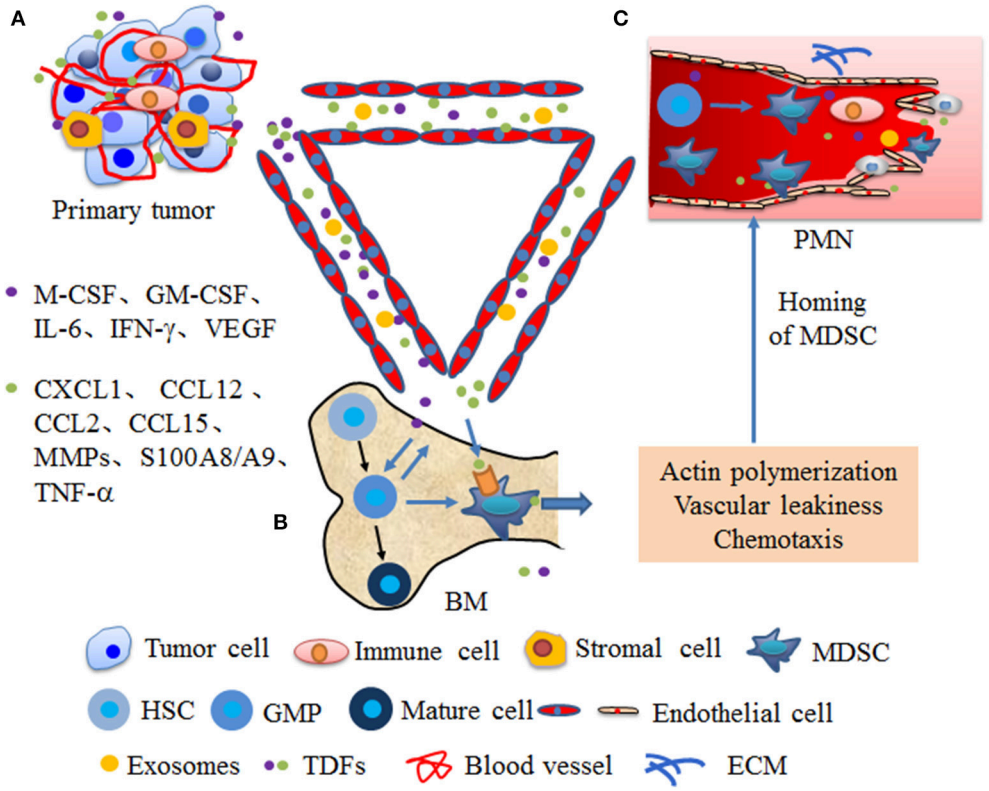

FIGURE 1 | Primary tumors promote the mobilization of MDSCs from bone marrow to secondary sites. **(A)** At primary tumor site, tumor or stromal cells secrete numerous cytokines and EVs that are systemically distributed following the blood circulation. **(B)** In the bone marrow (BM), cytokines, such as macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin 6 (IL-6), interferon gamma (IFN-γ), vascular endothelial growth factor (VEGF) from primary tumors promote MDSCs differentiation from granulocyte/monocyte precursor (GMP). Moreover, these cytokines mobilize MDSCs into the bloodstream through enhancing actin polymerization and vascular leakiness. **(C)** Cytokines from primary tumors, such as chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (CC motif) ligand 12 (CCL12), chemokine (CC motif) ligand 2 (CCL2), chemokine (CC motif) ligand 15 (CCL15), matrix metalloproteinases (MMPs), S100A8/A9, and tumor necrosis factor α (TNF-α) guide the homing of MDSCs to in secondary sites through chemotaxis and enhancin vascular remodeling, which create conditions conducive for MDSC mobilization to PMN. Moreover, factors or exosomes from primary tumor also enhance progenitors mobilization to the PMN and these progenitors further differentiate into MDSCs.

MDSC expansion and PMN formation. Blocking PMN formation through targeting ECM remodeling-related cytokines is an outstanding opportunity that is awaiting further research.

PRIMARY MDSC-RELATED PRO-PMN FACTORS AND MECHANISMS

Factors and cellular targets that mediate the steps of PMN formation and evolution, such as vascular leakiness, stromal education and reprogramming in organotropic sites, BMDC education and recruitment, and angiogenesis, should be validated in more detail. Dissecting PMN formation and evolution first requires examination of the earliest changes occurring within distant tissues. Current findings have identified EREG, COX2, and MMPs, which reconstitute a multi-functional vascular remodeling programme that leads to a large inflow of molecules and cells (80, 81). MDSCs are significantly increased in the lungs of mice bearing mammary adenocarcinomas before tumor cell arrival (18). The mechanisms by which MDSCs mediate PMN formation and evolution in original or distant organs remain to be elucidated, although MDSCs clearly play an immunosuppressive role through secreting Arg-1, NOS2, IL-10, COX2, ROS, TGF-β, PGE2, and IDO, sequestrating active site cysteine, decreasing L-selectin expression, and many other pathways (32). Chemokines, cytokines, growth factors and EVs from MDSCs participate in multiple stages of PMN formation and evolution. Although the individual factors of these mediators are insufficient to develop the PMN, their combined abilities result in a profound increase in the sequential steps of PMN development. In the 4T1 mammary and LLC lung carcinoma models, enhanced expression of pro-metastatic proteins in MDSCs, such as Bv8, MMP9, S100A8 and S100A9, facilitates improved PMN formation, which supports more efficient tumor cell extravasation and proliferation (44). In melanoma cell-bearing mice, the interactions of MDSCs with epithelial cells (ECs) involve an increase in vascular permeability and degradation of tight junction proteins (82). Additionally, we review the roles of MDSCs in promoting PMN formation and evolution and the possible mechanisms (**Figure 2**).

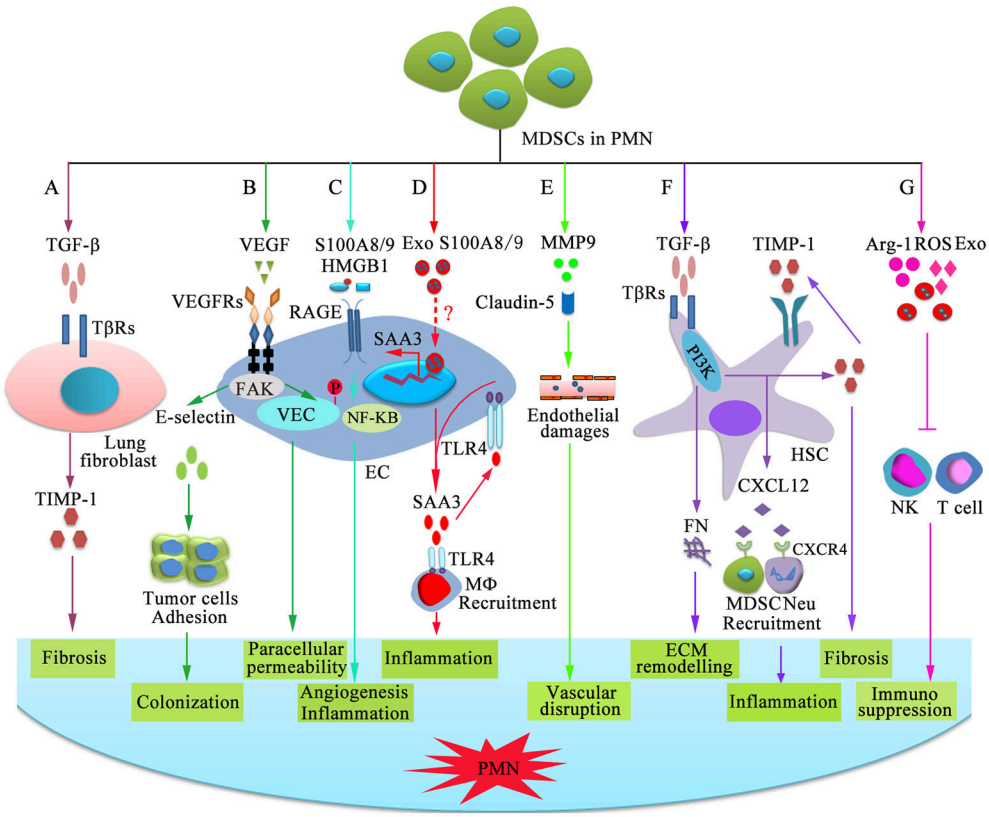

FIGURE 2 | Mechanisms of MDSC-dependent promotion of PMN formation and evolution. MDSC-derived factors participate in the stepwise evolution of the PMN through regulating local resident cells, resulting in a microenvironment that encourages the settlement and outgrowth of incoming cancer cells. **(A)** MDSCs stimulate lung fibroblasts to release tissue inhibitor of metalloproteinase 1 (TIMP1) by producing TGF-β, which promotes lung fibrosis. **(B)** VEGF-dependent induction of endothelial focal adhesion kinase (FAK) promotes E-selectin upregulation, which facilitates the adhesion of circulating tumor cells. VEGF triggers FAK-dependent vascular endothelial cadherin (VEC) phosphorylation in ECs and initiates paracellular permeability. **(C)** S100A8/9 and HMGB1 bind to RAGE on ECs and promote capillary-like tube formation and production of pro-inflammatory factor through the NF-κB signaling pathway, which is beneficial for angiogenesis and inflammation. **(D)** Exosomal S100A8/9 regulates SAA3 expression by ECs. SAA3 attracts macrophages to the pre-metastatic lungs through Toll-like receptor 4 (TLR4), which is beneficial for the formation of inflammatory microenvironment. **(E)** MMP9 damages the endothelial barrier of blood vessel through damaging tight junction protein claudin-5. **(F)** TGF-β induces fibronectin (FN) production and endogenous TIMP1 expression in hepatic stellate cells (HSCs) through phosphatidylinositol 3-kinase (PI3K). FN is conducive to tissue remodeling in the liver and initiate PMN formation. Moreover, circulating TIMP1-activated HSCs express C-X-C motif chemokine 12 (CXCL12), which induces MDSC and neutrophil migration through CXCR4 and creates a microenvironment in the liver that increases its susceptibility to tumor cells. **(G)** MDSCs suppress NK-and T-cell function by secreting immunosuppressive molecules and exosomes.

TGF-β

TGF-β is a secreted polypeptide that is a key element of cancer progression toward metastasis. In colon and breast cancer mouse models, TGF-β assists in the whole metastatic dissemination process through crosstalk with cancer cells, cancer-associated fibroblasts and immune cells, which contribute to the process (83, 84). During PMN formation and evolution, MDSCs are one important source of TGF-β, which induces a series of pre-metastatic events. Exposure of pulmonary tissue to single-walled carbon nanotubes leads to TGF-β production by MDSCs, which favors the formation of a microenvironment that supports ingrowth of lung carcinoma cells (85). However, the role of TGF-β signaling in MDSC-mediated PMN formation and evolution is unclear. In the lung, $CCR2^{+}$ M-MDSCs stimulate lung fibroblasts to release tissue inhibitor of metalloproteinase 1 (TIMP1) by producing TGF-β, which promotes lung fibrosis (86). In the liver, high TIMP1 protein levels in premalignant pancreatic lesions induce endogenous TIMP1 expression in hepatic stellate cells (HSCs) through interaction with CD63 and a process that involves the phosphatidylinositol 3-kinase (PI3K) molecule. Moreover, circulating TIMP1-activated HSCs express C-X-C motif chemokine 12 (CXCL12), which induces MDSC and neutrophil migration through CXCR4 and creates a microenvironment in the liver that increases its susceptibility to pancreatic tumor cells (57). Moreover, chronic inflammation activates human HSCs also to convert mature peripheral blood monocytes into MDSCs in a CD44-dependent fashion (87).In addition, TGF-β secretion from Kupffer cells promotes the upregulation of fibronectin production by HSCs in a pancreatic cancer mouse model, which recruit macrophages into the liver and initiate PMN formation (43). In patients with non-small cell lung cancer, TGF-β stimulates CD39 and CD73

expression on MDSCs in the PMN and inhibits T cell and NK cell activity (88). These results indicate that MDSCs may promote PMN formation through TGF-β protein secretion. Therefore, TGF-β may be an effective target for suppression of PMN formation.

VEGF

The study of PMN formation largely focused on the lung as a target organ. In fact, the pre-metastatic lung contains many hyperpermeable vessels, activated endothelial cells, and abundant E-selectin (89, 90). The tumor microvasculature tends to be malformed, more permeable, and more tortuous than vessels in healthy tissue. These effects have been largely attributed to upregulated VEGF expression (91). In esophageal squamous cell carcinoma patients, endothelial cells within the hyperpermeable area of the PMN have been proposed to produce TGF-β in a paracrine manner, leading to fibroblast activation and VEGF release (92). Interestingly, VEGF triggers focal adhesion kinase (FAK)-dependent vascular endothelial cadherin (VEC) tyrosine (Y) 658 (VEC-Y658) phosphorylation in ECs and initiates paracellular permeability (82). Moreover, VEGFA-dependent induction of endothelial FAK or injection of recombinant VEGFA promotes E-selectin upregulation, and inhibition of endothelial cell FAK hinders lung metastasis (82, 90). E-selectin facilitates the adhesion of circulating tumor cells and lead to preferential homing of metastatic cancer cells to these foci and outgrowth. In tumor-bearing mice transplanted with B16F1, Tib6, EL4, or LLC cells, G-CSF secretion by the tumor mobilizes $CD11b^{+}Gr1^{+}$ myeloid cells to secrete VEGFA, which affects the tumor vasculature and promotes the formation of a pre-metastatic lung microenvironment (63). Overall, VEGF from MDSCs promotes PMN formation directly or indirectly and therefore may be candidate for blocking PMN.

S100A8/9

A common denominator of inflammatory responses within the PMN is the S100 protein. The pro-inflammatory mediator S100A8/A9 is abundant at inflammatory sites. S100A8/A9 is involved in processes such as enhancement of Ca^{2+} influx, cytokine production, immune cell recruitment and inflammation (93). The exact mechanism of S100A8/S100A9 in PMN formation is unclear, although S100A8/S100A9 is crucial for intercellular crosstalk between tumor and stromal cells during PMN establishment. Extracellular S100A8/A9 from MDSCs and tumor cells stimulates macrophage polarization toward the tumor-promoting M2 phenotype, and this conversion switches off IL-12 production, which drives the development of NK cells and tumouricidal T lymphocytes (94). Moreover, S100A8/S100A9 from mammary carcinoma cells bind to RAGE on MDSCs and promote the migration and accumulation of MDSCs through the NF-κB signaling pathways (77). In addition, secretion of S100A8/S100A9 proteins by MDSCs activates endothelial cells and MDSCs, resulting in myeloid cell recruitment in the blood and secondary lymphoid organs (77). S100 proteins and high mobility group box-1 protein (HMGB1) secreted by MDSCs are ligands of RAGE. Downstream signaling pathways of RAGE are expressed in endothelial cells and MDSCs. HMGB1 effectively promotes human pulmonary microvascular endothelial cell migration and capillary-like tube formation through the ERK/P38/Src signaling pathway (95). Thus, S100A8/S100A9 may maintain an autocrine feedback loop that leads to MDSC recruitment within the PMN.

Interestingly, human breast cancer cell-derived exosomes prepare the PMN by activating Src phosphorylation and pro-inflammatory S100 gene expression in organ-specific cells (42). Moreover, MDSC exosomes induce chemotaxis of MDSCs themselves through their S100A8 and A9 content and promote M2 macrophage polarization in breast cancer model mice (96). In pancreatic cancer model mice, TGF-β signaling-induced fibronectin (FN) upregulation induces macrophage recruitment to the liver, which promotes liver PMN formation (43). Upon CCL2 stimulation, exosomal S100A8/9 produced by primary LLC or B16 cells are delivered systemically to the pre-metastatic lung endothelium and regulate SAA3 expression by endothelial cells and alveolar macrophages through stimulating the SAA3 promoter, which attracts $CD11b^{+}$ myeloid cells to the pre-metastatic lungs (65). Furthermore, SAA3 also binds to TLR4 on lung endothelial cells and macrophages (65). Pancreatic cancer cell-derived exosomes initiate PMN formation in the liver through macrophage migration inhibitory factor (MIF) (43). This S100A8-SAA3-TLR4 cascade establishes the PMN. These results suggest that exosomal S100A8/9 play an important role in PMN formation, although the exact mechanism remains to be clarified.

MMP9

MMPs from invasive endothelial cells or bone marrow derived-progenitor cells govern degradation of the extracellular matrix, basement membrane, and interstitial stroma, all of which are essential events during the formation of new blood vessels (91). MMP9 is a member of a family of zinc-containing endopeptidases and is maintained at high levels in the PMN (72, 97). Activation of endothelial MMP9 leads to damage of the endothelial barrier (98). Inhibiting MMP9 activity can decrease vascular permeability and improve stroke (99). Moreover, in a breast cancer mouse model, altered vascular integrity is manifested through hyperpermeability in the PMN, aberrant morphology of the vascular endothelium and breakdown of the vascular basement membrane (89). MMP9 also damaged vessel stability through sequestering vascular VEGF and TGF-β in the ECM (100). In fact, vascular permeability and neo-angiogenesis generation favor the initial extravasation and subsequent metastatic growth of tumor cells into pre-metastatic organs (101). MMP9 plays crucial roles in ECM remodeling and the angiogenic switch that supports formation of the PMN (102). MMP9 produced by MDSCs causes abnormal and leaky vasculature as well as restructuring of collagen in the basement membrane of blood vessels in the pre-metastatic lung (103). In mammary adenocarcinoma-bearing mice, $CD11b^{+}Gr\text{-}1^{+}$ myeloid progenitor cells are significantly increased in the pre-metastatic lung before tumor cell arrival and produce a large amount of MMP9, which

promotes aberrant vasculature formation and leads to the formation of a proliferative, immunosuppressive and inflamed PMN in the lung (18). Moreover, ablation of MMP9 results in aberrant vasculature normalization, improvement of host immune surveillance, and diminished lung metastasis (18). Therefore, MMP9 is a crucial regulator of mobilization of bone marrow-derived endothelial cell and progenitor cell recruitment, ECM remodeling, and the angiogenic switch, which are intimately involved in regulating vascular integrity in the PMN. These results suggest that MMP9 from MDSCs or tissue-resident cells in the pre-metastatic lung destroys the vasculature stability and immune balance, resulting in PMN formation and evolution.

EXOSOMES IN MDSC RECRUITMENT AND PMN FORMATION

Exosomes (30–150 nm) are one type of membrane vesicle of endocytic origin and are secreted into the extracellular space by most cell types. Exosomes perform many biological functions, particularly intercellular communication through delivering functional proteins, mRNAs, and miRNAs into target cells following the internalization of exosomes. Cumulative evidence has suggested that tumor exosomes fuse with resident cells in the PMN and transfer their cargo, including genetic material (DNA, mRNA and miRNA), metabolites (lipids and small metabolites) and proteins, which are closely associated with the initiation, formation, and evolution of the PMN (101, 104, 105).

Exosomes shed by tumor cells have been shown to contribute to MDSC recruitment. For example, membrane-associated Hsp72 from colon carcinoma CT26, lymphoma EL4, and embryo fibroblast NIH/3T3 cells and human lung adenocarcinoma-derived exosomes mediates the STAT3-dependent immunosuppressive function of MDSCs (58). Another study show that melanoma cell-derived exosomes promote PMN formation by educating bone marrow progenitor cells toward a pro-metastatic phenotype through the MET protein (12) (**Table 2**). Pancreatic tumor-derived exosomes expressing MIF promote TGF-β secretion from Kupffer cells, which stimulates fibronectin secretion from hepatic stellate cells and recruits myeloid $CD11b^+$ cells to the PMN in the liver (43). The pro-inflammatory proteins S100A8/S100A9 are abundant in MDSC exosomes from breast cancer model mice and is chemotactic for MDSCs *in vitro* (96). Therefore, exosomes from primary tumors play important roles in MDSC recruitment in secondary organ. The blockade of critical exosomes or their cargo is beneficial for inhibiting the accumulation and activation of MDSCs in the PMN.

Exosomes enhance the systematic entry of cancer cells along the metastatic cascade. Therefore, understanding the biology of MDSC exosomes in the PMN is important. Mass spectrometry results show that MDSC exosomes from breast cancer model mice carry biologically active components, such as metabolic enzymes, transcription factors, and proteins relevant for immunomodulation (96). MDSC exosomes also carry many surface glycoproteins and several shared ligand receptor pairs, indicating that MDSC exosomes are well equipped for binding (106). In the following paragraphs, we will further examine the possible roles of MDSC exosomes in diverse mechanisms related to PMN formation and evolution, which are favorable for inhibiting PMN establishment at secondary organs and consequent metastatic outgrowth.

The integrin on the surface of breast cancer cell exosomes promotes immature myeloid cell homing to the PMN and increases activation of S100 genes and Src signaling in the PMN in the lung and liver (7). LLC or B16/F10 cell-derived exosomal RNA activates alveolar epithelial TLR3 and consequently induces chemokine secretion in the lung and promotes neutrophil recruitment, which also promotes lung PMN formation (104). Therefore, the interactions of MDSC exosomes and cargo with ECs need to be clarified further. In cancer patients, intratumoural and peripheral MDSCs inevitably shed large exosomes, which are involved in PMN formation and evolution, although the exact mechanism needs to be further clarified. Breast cancer cell exosomal miR-210 promotes angiogenesis and metastasis by regulating EC behavior (107, 108). Interestingly, HIF-1α can induce miR-210 overexpression in MDSCs and increase arginase activity and nitric oxide production (108), although miR-210 expression in MDSC exosomes needs to be further clarified. A study showed that MDSC exosomal miR-126a promoted lung metastasis by breast tumors (38) (**Table 3**). Moreover, melanoma exosomal miR-9 activates the JAK-STAT pathway through reducing the SOCS5 levels in ECs, which promotes endothelial cell migration and tumor angiogenesis (126). CREB regulates miR-9 expression and inhibits MDSC differentiation by targeting runt-related transcription factor 1 (RUNX1) (24). The miR-9 expression profile in MDSC exosomes needs to be identified, and the interactions between miR-9 and ECs need to be further investigated. MDSCs express the advanced glycosylation end-product-specific receptor ligands S100A8/9, which can contribute to activation of inflammatory/immunosuppressive genes. MDSC exosomes polarize macrophages toward a tumor-promoting type 2 phenotype and possess S100A8/A9 chemotactic activity (96). G-MDSC exosomal Arg-1 inhibits T cell proliferation (127). Clearly, many cargoes within MDSC exosomes participate in function modulation and metabolic reprogramming of immune and stromal cells.

These results indicate that MDSC exosomes are favorable for the establishment of an inflammatory and immunosuppressive microenvironment that is a supportive niche for the arrival of tumor cells. The potential impact of MDSC exosomes on regulation of the PMN is definite, although the detailed mechanism still needs further exploration.

POTENTIAL APPLICATION OF MDSCS IN PMN DETECTION AND THERAPY

Clinical establishment of PMN detection technology could help patients optimize the selection of monitoring and intervention during therapy. Nevertheless, no effective clinical techniques are available to detect the PMN at present. Early detection

TABLE 3 | Molecules associated with the blockade of MDSC expansion and recruitment.

Molecules	Cancer type	Phenotype	Species	References
VITAMIN DERIVATIVES				
1α,25-hydroxy vitamin D3	HNSCC	MDSCs	Human	(109)
ATRA	Fibrosarcomas	MDSCs	Mouse	(110)
	Mammary adenocarcinomas	MDSCs	Mouse	(110)
	Renal cell carcinoma	MDSCs	Human	(111)
Vitamin D	CLL	$CD14^{+}HLA\text{-}DR^{low}$ MDSCs	Human	(112)
AMINO-BISPHOSPHONATE				
ZA	Mesothelioma	MDSCs	Mouse	(113)
	Myeloma	MDSCs	Mouse	(114)
	Pancreatic cancer	$CD15^{+}CD11b^{+}$ MDSCs	Human	(115)
	Pancreatic cancer	MDSCs	Mouse	(115)
	Breast cancer	MDSCs	Mouse	(116)
ANTIBODIES				
Anti-VEGFR-2 Ab	Melanoma and prostate tumor	M-MDSCs	Mouse	(117)
Anti-Gr1 Ab	Lung cancer	MDSCs	Mouse	(118)
	Myeloma	MDSCs	Mouse	(119)
MD5-1 mAb	Lymphoma	MDSCs	Mouse	(120)
DS-8273a mAb	Advanced cancers	MDSCs	Human	(121)
Anti-CD33 Ab	Myelodysplastic syndrome	$CD33^{+}HLA\text{-}DR^{-}Lin^{-}$ MDSCs	Human	(122)
Anti-KIT mAb	Colon cancer	M-MDSCs	Mouse	(123)
Anti-ENO1 mAb	Pancreatic ductal adenocarcinoma	MDSCs	Mouse	(124)
Anti-DC-HIL mAb	Colorectal cancer	M-MDSCs	Mouse	(125)

of the PMN before radiographic evidence of the metastatic niche remains a challenge. Following immune cells or related molecules using a radiographic method provides an opportunity to identify the PMN. For instance, whole body imaging of lymphovascular niches is used to identify the premetastatic roles of melanoma in mice (128). However, the lack of specific tracking probes hinders the application of positron emission tomography (PET) and nuclear magnetic resonance (NMR) for PMN detection. Considering the crucial role of MDSCs in pre-metastatic tissue priming and the abundance of S100A8/A9 in MDSCs, initiation of the PMN can most likely be predicted by the MDSC abundance, which is reflected by MSDC surface molecules or cytokines. Researchers have developed a method that uses antibody-based single-photon emission computed tomography (SPECT) for detection of S100A8/A9 *in vivo* as an imaging marker for pre-metastatic tissue priming (20). However, because MDSCs are not the only source of S100A8/A9, more MDSC-related molecules should be tested. Published studies have proven the roles of exosome-mediated PMN formation with diverse mechanisms.

Study showed that pancreatic cancer cell-derived exosomes initiated PMN formation in the liver through MIF (43). Moreover, human breast cancer cell-derived exosomal integrins (ITGs) direct organ-specific colonization by fusing with resident target cells in a tissue-specific fashion, thereby initiating PMN formation (7). Those tumor exosomal cargoes in plasma assist with the diagnosis and prognostic assessment of the corresponding diseases. However, those tumor exosomal cargoes play a limited role in PMN detection, because there is no effective tracer for these molecules and their distribution profiles in the pre-metastatic microenvironment are unclear. MDSC exosomes package various molecules, including S100A8/9 (96), miR-126a (38), and Arg-1 (127), which are involved in PMN formation and evolution. Moreover, MDSC exosomes express CD11b molecules (106), which provide the possibility for an exosome trace. Therefore, MDSC exosomes have potential application value for detection of the PMN.

Currently, no clinical agents are a specific target therapy for the PMN, although targeted therapies directed against establishment of the PMN can potentially inhibit metastasis in mice. In the earliest PMN event, ECM remodeling and the formation of blood clots lead to the loss of vascular integrity, which causes increased vasculature permeability. In turn, the increased vasculature permeability is beneficial for the ability of macromolecules and cells to cross endothelial barriers, which leads to ECM remodeling and destruction of vascular integrity. On the other hand, vascular leakiness leads to an abnormal microenvironment that is characterized by interstitial hypertension (elevated hydrostatic pressure outside the blood vessels). Therefore, targeting drugs to the PMN is difficult due to the increased permeability of the vasculature at the PMN (19). Encouragingly, specific targeting of PMN components reduces metastasis in preclinical models. In breast cancer, inhibition of LOX activity abrogates the formation of tumor-driven focal pre-metastatic bone lesions (129). In mice, the formation of pre-metastatic cellular clusters can be

abrogated by preventing VEGFR1 function using antibodies or by removing VEGFR1$^+$ cells from the bone marrow (4). Blocking SAA3-TLR4 function during the pre-metastatic phase can prevent formation of the pulmonary PMN (65). Abrogation of HPC clusters within pre-metastatic organs by either a VEGFR1 antibody or depletion of VEGFR1$^+$ BMDCs reduces the metastasis of LLC or B16 cells to lung tissue (4). MDSCs play a crucial role in PMN formation and evolution and present strategic therapeutic potential. The use of low doses of approved chemotherapeutic drugs, such as 5-fluorouracil, gemcitabine, and fludarabine, represents the most promising, and feasible strategy to reduce the intratumoural numbers of MDSCs (130). In addition, small molecules, such as vitamin derivatives (112), amino-bisphosphonate (113), and antibodies (117), have been found to block MDSC expansion and recruitment (**Table 3**). Therefore, strategies to eliminate MDSCs and their related molecules and exosomes will help prevent PMN formation.

Vitamin Derivatives

Vitamins A and D may aid in MDSC differentiation to more mature cells through an unknown mechanism, which has been reviewed (131, 132). The efficacy of 1α, 25-hydroxyvitamin D3 was observed in mice with lung cancer and patients with non-small cell lung and squamous cell carcinoma of the head and neck (109, 133, 134). In addition, all-trans-retinoic acid (ATRA) is a derivative of vitamin A with antiproliferative properties. ATRA targets genes responsible for cell maturation that are less likely to favor tumor growth by maturing MDSCs into DCs, granulocytes, and monocytes (135). In mice with fibrosarcomas and mammary adenocarcinomas, ATRA also enhance antitumour T cell responses (110). Clinical trials have shown that renal cell carcinoma patients with high serum ATRA concentrations have fewer peripheral blood MDSCs and improved T cell responses (111). Some new discoveries have been made concerning the regulation of MDSCs by vitamin derivatives. miR-155 induces MDSC expansion via targeting SH2 domain-containing inositol 5′-phosphatase 1 (SHIP1), leading to STAT3 activation (136). In B cell-derived chronic lymphocytic leukemia (CLL), transfer of tumor cell exosomal miR-155 contributes to CLL cell-mediated MDSC induction, which can be disrupted by vitamin D (112). Last but not least, in a model of lipopolysaccharide-induced immunosuppression, ATRA decreases the generation of MDSCs by reducing CD34$^+$ precursor cell proliferation (137). Therefore, vitamin derivatives may be candidates for blocking PMN and need to be thoroughly studied through clinical trials.

Amino-Bisphosphonate

Amino-bisphosphonate has been suggested to work as an immune modulator and therefore may be applicable as an antitumour agent that can prolong disease-free survival in cancer patients. Zoledronic acid (ZA) is a potent amino-bisphosphonate that targets the mevalonate pathway in myeloid cells. Zoledronic acid was previously shown to target MDSCs. In mesothelioma, ZA suppress TAM differentiation from MDSCs, leading to a reduced level of TAM-associated cytokines in the tumor microenvironment (113). In myeloma-challenged mice, ZA inhibits the expansion of MDSCs and bone lesions (114). In pancreatic cancer mice, ZA impairs intratumoural MDSC accumulation, resulting in a delayed tumor growth rate, prolonged median survival, and increased recruitment of T cells to the tumor (115). Amino-bisphosphonates contribute to specific MMP-9 inhibitory activity (116). In mammary tumor model mice, amino-bisphosphonates significantly reduce MDSC expansion in both the bone marrow and peripheral blood by decreasing the serum pro-MMP-9 and VEGF levels (116). These studies reinforce the importance of amino-bisphosphonate t in preventing the PMN formation and evolution.

Antibodies

Antibodies are widely used as efficient agents for eliminating MDSCs, although their efficacies for each MDSC subtype (G-MDSCs and M-MDSCs) are controversial. For example, in melanoma and prostate tumor model mice, an anti-VEGFR-2 antibody suppresses MDSC-mediated angiogenesis through MMP-9 inhibition (117). Moreover, an anti-Gr1 antibody (RB6-8C5) is widely used as an efficient agent to eliminate MDSCs in mice. Zhang et al. (118) found that an anti-Gr1 antibody reduced MDSCs by one-third in the tumors of 3LL cell-bearing mice. Vincent Hurez used an anti-Gr1 monoclonal antibody that reduced MDSCs by 50–75% in the spleens of B16-bearing mice (119). In addition, MDSCs are sensitive to TNF-related apoptosis–induced ligand receptor 2 (TRAIL-R2) agonists. DR5, which is a TRAIL-R, plays an important role in MDSC survival. The MD5-1 mAb, which is an agonistic DR5 antibody, dramatically improves immune responses in tumor-bearing mice. In mice bearing large EL4 tumors, treatment with the MD5-1 mAb strongly decreases the accumulation of both MDSC subsets in the tumor, and this effect is quite specific for MDSCs without affecting DCs and macrophages (120). In 16 patients with advanced cancers, the agonistic TRAIL-R2 antibody DS-8273a selectively targeted MDSCs and resulted in reduction of the elevated numbers of MDSCs in the peripheral blood of most patients (121). In myelodysplastic syndrome, BI 836858, which is a Fc-engineered monoclonal antibody against CD33, also reduce MDSCs by antibody-dependent cellular cytotoxicity and block CD33 downstream signaling, thereby preventing immunosuppressive cytokine secretion (122). In colon 26 cell-bearing mice, the anti-KIT IgG1 mAb KTN0158 promoted immune responses by selectively reducing immunosuppressive M-MDSCs (123). In pancreatic ductal adenocarcinoma-bearing mice, a mAb targeting pancreatic ductal adenocarcinoma-associated antigen α-enolase (ENO1) inhibited *in vivo* infiltration of MDSCs into the tumor microenvironment and attenuated their restraint of the effector T cell response (124). Last but not least, in colorectal cancer with high blood DC-HIL$^+$ MDSC levels, an anti-DC-HIL mAb attenuated tumor progression by reducing MDSCs in the tumor microenvironment (125). These works provide a foundation for the development of a novel group of therapies for the PMN aimed at MDSCs. The combined use of these antibodies may more effectively

prevent the formation and evolution of PMN through targeting MDSCs.

CONCLUSIONS AND PERSPECTIVES

Taken together, the presented findings show that MDSC-derived TGF-β, S100A8/A9, VEGF, and exosomes promote PMN formation and metastasis through crosslinking with the immune system, fibroblasts, endothelial cells, and hepatic stellate cells. The main processes and mechanisms involve the induction of vascular leakiness, ECM remodeling, immunosuppression, and inflammation, although the exact mechanism remains to be confirmed. Because MDSCs play pivotal roles in PMN formation and evolution, developing strategies based on MDSCs for detection of the PMN at its earliest stages is realistic. Understanding the cross-talk between MDSCs and resident cells in pre-metastatic organs is essential for PMN targeting. Most of the work exploring PMN formation relies on mouse models of metastasis, and our understanding of PMN biology is mostly based on studies of lung or liver metastases. Some obstacles remain for clarifying the clinical traits of PMN and obtaining premetastatic tissues from patients. More clinical research is needed, and better imaging techniques for PMN detection should be developed.

AVAILABILITY OF DATA AND MATERIAL

The material supporting the conclusion of this review has been included within the article.

AUTHOR CONTRIBUTIONS

YW and SW designed the study. YW and NG drafted the manuscript. YD prepared the tables and figures. All authors read and approved the final manuscript.

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REFERENCES

1. Paget S. The distribution of secondary growths in cancer of the breast. *Cancer Metastasis Rev*. (1989) 8:98–101.
2. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell* (2006) 127:679–95. doi: 10.1016/j.cell.2006.11.001
3. Poste G, Fidler IJ. The pathogenesis of cancer metastasis. *Nature* (1980) 283:139–46.
4. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* (2005) 438:820–7. doi: 10.1038/nature04186
5. Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, et al. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* (2002) 2:289–300. doi: 10.1016/S1535-6108(02)00153-8
6. Tichet M, Prod'Homme V, Fenouille N, Ambrosetti D, Mallavialle A, Cerezo M, et al. Tumour-derived SPARC drives vascular permeability and extravasation through endothelial VCAM1 signalling to promote metastasis. *Nat Commun*. (2015) 6:6993. doi: 10.1038/ncomms7993
7. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. *Nature* (2015) 527:329–35. doi: 10.1038/nature15756
8. Yang WW, Yang LQ, Zhao F, Chen CW, Xu LH, Fu J, et al. Epiregulin promotes lung metastasis of salivary adenoid cystic carcinoma. *Theranostics* (2017) 7:3700–14. doi: 10.7150/thno.19712
9. Zhang C, Zhou C, Wu XJ, Yang M, Yang ZH, Xiong HZ, et al. Human CD133-positive hematopoietic progenitor cells initiate growth and metastasis of colorectal cancer cells. *Carcinogenesis* (2014) 35:2771–7. doi: 10.1093/carcin/bgu192
10. Zhang Y, Davis C, Ryan J, Janney C, Pena MM. Development and characterization of a reliable mouse model of colorectal cancer metastasis to the liver. *Clin Exp Metastasis* (2013) 30:903–18. doi: 10.1007/s10585-013-9591-8
11. Wong CC, Gilkes DM, Zhang H, Chen J, Wei H, Chaturvedi P, et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. *Proc Natl Acad Sci USA*. (2011) 108:16369–74. doi: 10.1073/pnas.1113483108
12. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med*. (2012) 18:883–91. doi: 10.1038/nm.2753
13. Seubert B, Grunwald B, Kobuch J, Cui H, Schelter F, Schaten S, et al. Tissue inhibitor of metalloproteinases (TIMP)-1 creates a premetastatic niche in the liver through SDF-1/CXCR4-dependent neutrophil recruitment in mice. *Hepatology* (2015) 61:238–48.doi: 10.1002/hep.27378
14. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci USA*. (2010) 107:21248–55. doi: 10.1073/pnas.1015855107
15. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci USA*. (2015) 112:E566–75. doi: 10.1073/pnas.1424927112
16. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell* (2011) 20:300–14. doi: 10.1016/j.ccr.2011.08.012
17. Lavender N, Yang J, Chen SC, Sai J, Johnson CA, Owens P, et al. The Yin/Yan of CCL2: a minor role in neutrophil anti-tumor activity *in vitro* but a major role on the outgrowth of metastatic breast cancer lesions in the lung *in vivo*. *BMC Cancer* (2017) 17:88. doi: 10.1186/s12885-017-3074-2
18. Yan HH, Pickup M, Pang Y, Gorska AE, Li Z, Chytil A, et al. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res*. (2010) 70:6139–49. doi: 10.1158/0008-5472.can-10-0706
19. Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, et al. Pre-metastatic niches: organ-specific homes for metastases. *Nat Rev Cancer* (2017) 17:302–17. doi: 10.1038/nrc.2017.6
20. Eisenblaetter M, Flores-Borja F, Lee JJ, Wefers C, Smith H, Hueting R, et al. Visualization of tumor-immune interaction - target-specific imaging of S100A8/A9 reveals pre-metastatic niche establishment. *Theranostics* (2017) 7:2392–401. doi: 10.7150/thno.17138

21. Sceneay J, Parker BS, Smyth MJ, Moller A. Hypoxia-driven immunosuppression contributes to the pre-metastatic niche. *Oncoimmunology* (2013) 2:e22355. doi: 10.4161/onci.22355
22. Giles AJ, Reid CM, Evans JD, Murgai M, Vicioso Y, Highfill SL, et al. Activation of hematopoietic stem/progenitor cells promotes immunosuppression within the pre-metastatic niche. *Cancer Res.* (2016) 76:1335–47. doi: 10.1158/0008-5472.can-15-0204
23. Owyong M, Efe G, Owyong M, Abbasi AJ, Sitarama V, Plaks V. Overcoming barriers of age to enhance efficacy of cancer immunotherapy: the clout of the extracellular matrix. *Front Cell Dev Biol*. (2018) 6:19. doi: 10.3389/fcell.2018.00019
24. Tian J, Rui K, Tang X, Ma J, Wang Y, Tian X, et al. MicroRNA-9 Regulates the differentiation and function of myeloid-derived suppressor cells via targeting Runx1. *J Immunol*. (2015) 195:1301–11. doi: 10.4049/jimmunol.1500209
25. Tian J, Rui K, Hong Y, Wang X, Xiao F,Lin X, et al. Increased GITRL impairs the function of myeloid-derived suppressor cells and exacerbates primary Sjögren's syndrome. *J Immunol.* (2019). doi: 10.4049/jimmunol.1801051. [Epub ahead of print].
26. Zhao Y, Wu T, Shao S, Shi B, Zhao Y. Phenotype, development, and biological function of myeloid-derived suppressor cells. *Oncoimmunology* (2016) 5:e1004983. doi: 10.1080/2162402x.2015.1004983
27. Zhou Q, Tang X, Tian X, Tian J, Zhang Y, Ma J, et al.LncRNA MALAT1 negatively regulates MDSCs in patients with lung cancer. *J Cancer* (2018) 14:2436–42. doi: 10.7150/jca.24796
28. Kwak HJ, Liu P, Bajrami B, Xu Y, Park SY, Nombela-Arrieta C, et al. Myeloid cell-derived reactive oxygen species externally regulate the proliferation of myeloid progenitors in emergency granulopoiesis. *Immunity* (2015) 42:159–71. doi: 10.1016/j.immuni.2014.12.017
29. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* (2007) 450: 825–31. doi: 10.1038/nature06348
30. Maenhout SK, Thielemans K, Aerts JL. Location, location, location: functional and phenotypic heterogeneity between tumor-infiltrating and non-infiltrating myeloid-derived suppressor cells. *Oncoimmunology* (2014) 3:e956579. doi: 10.4161/21624011.2014.956579
31. Safarzadeh E, Orangi M, Mohammadi H, Babaie F, Baradaran B. Myeloid-derived suppressor cells: Important contributors to tumor progression and metastasis. *J Cell Physiol*. (2018) 233:3024–36. doi: 10.1002/jcp.26075
32. Wang Y, Tian J, Wang S. The potential therapeutic role of myeloid-derived suppressor cells in autoimmune arthritis. *Semin Arthritis Rheum*. (2016) 45:490–5. doi: 10.1016/j.semarthrit.2015.07.003
33. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
34. Solito S, Pinton L, Mandruzzato S. In Brief: Myeloid-derived suppressor cells in cancer. *J Pathol.* (2017) 242:7–9. doi: 10.1002/path.4876
35. Shojaei F, Zhong C, Wu X, Yu L, Ferrara N. Role of myeloid cells in tumor angiogenesis and growth. *Trends Cell Biol*. (2008) 18:372–8. doi: 10.1016/j.tcb.2008.06.003
36. Cui TX, Kryczek I, Zhao L, Zhao E, Kuick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity* (2013) 39:611–21. doi: 10.1016/j.immuni.2013.08.025
37. Toh B, Wang X, Keeble J, Sim WJ, Khoo K, Wong WC, et al. Mesenchymal transition and dissemination of cancer cells is driven by myeloid-derived suppressor cells infiltrating the primary tumor. *PLoS Biol*. (2011) 9:e1001162. doi: 10.1371/journal.pbio.1001162
38. Deng Z, Rong Y, Teng Y, Zhuang X, Samykutty A, Mu J, et al. Exosomes miR-126a released from MDSC induced by DOX treatment promotes lung metastasis. *Oncogene* (2017) 36:639–51. doi: 10.1038/onc.2016.229
39. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of Myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol.* (2016) 37:208–20. doi: 10.1016/j.it.2016.01.004
40. Tian X, Ma J, Wang T, Tian J, Zhang Y, Mao L, et al. Long non-coding RNA HOXA transcript antisense RNA Myeloid-specific 1-HOXA1 axis downregulates the immunosuppressive activity of Myeloid-derived suppressor cells in lung cancer. *Front Immunol.* (2018) 9:473. doi: 10.3389/fimmu.2018.00473
41. Haverkamp JM, Smith AM, Weinlich R, Dillon CP, Qualls JE, Neale G, et al. Myeloid-derived suppressor activity is mediated by monocytic lineages maintained by continuous inhibition of extrinsic and intrinsic death pathways. *Immunity* (2014) 41:947–59. doi: 10.1016/j.immuni.2014.10.020
42. Aguado BA, Caffe JR, Nanavati D, Rao SS, Bushnell GG, Azarin SM, et al. Extracellular matrix mediators of metastatic cell colonization characterized using scaffold mimics of the pre-metastatic niche. *Acta Biomater.* (2016) 33:13–24. doi: 10.1016/j.actbio.2016.01.043
43. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol*. (2015) 17:816–26. doi: 10.1038/ncb3169
44. Wu CF, Andzinski L, Kasnitz N, Kroger A, Klawonn F, Lienenklaus S, et al. The lack of type I interferon induces neutrophil-mediated pre-metastatic niche formation in the mouse lung. *Int J Cancer* (2015) 137:837–47. doi: 10.1002/ijc.29444
45. Murgai M, Ju W, Eason M, Kline J, Beury DW, Kaczanowska S, et al. KLF4-dependent perivascular cell plasticity mediates pre-metastatic niche formation and metastasis. *Nat Med*. (2017) 23:1176–90. doi: 10.1038/nm.4400
46. Casacuberta-Serra S, Pares M, Golbano A, Coves E, Espejo C, Barquinero J. Myeloid-derived suppressor cells can be efficiently generated from human hematopoietic progenitors and peripheral blood monocytes. *Immunol Cell Biol*. (2017) 95:538–48. doi: 10.1038/icb.2017.4
47. Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). *Clin Cancer Res*. (2016) 22:3924–36. doi: 10.1158/1078-0432.ccr-15-2463
48. Horikawa N, Abiko K, Matsumura N, Hamanishi J, Baba T, Yamaguchi K, et al. Expression of vascular endothelial growth factor in ovarian cancer inhibits tumor immunity through the accumulation of myeloid-derived suppressor cells. *Clin Cancer Res.* (2017) 23:587–99. doi: 10.1158/1078-0432.ccr-16-0387
49. Hart KM, Usherwood EJ, Berwin BL. CX3CR1 delineates temporally and functionally distinct subsets of myeloid-derived suppressor cells in a mouse model of ovarian cancer. *Immunol Cell Biol*. (2014) 92:499–508. doi: 10.1038/icb.2014.13
50. Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proc Natl Acad Sci USA*. (2017) 114:1117–22. doi: 10.1073/pnas.1612920114
51. Ridder K, Sevko A, Heide J, Dams M, Rupp AK, Macas J, et al. Extracellular vesicle-mediated transfer of functional RNA in the tumor microenvironment. *Oncoimmunology* (2015) 4:e1008371. doi: 10.1080/2162402x.2015.1008371
52. Hsieh CC, Chou HS, Yang HR, Lin F, Bhatt S, Qin J, et al. The role of complement component 3 (C3) in differentiation of myeloid-derived suppressor cells. *Blood* (2013) 121:1760–8. doi: 10.1182/blood-2012-06-440214
53. Wong JL, Obermajer N, Odunsi K, Edwards RP, Kalinski P. Synergistic COX2 Induction by IFNgamma and TNFalpha self-limits type-1 immunity in the human tumor microenvironment. *Cancer Immunol Res*. (2016) 4:303–11. doi: 10.1158/2326-6066.cir-15-0157
54. Hossain DM, Pal SK, Moreira D, Duttagupta P, Zhang Q, Won H, et al. TLR9-targeted STAT3 silencing abrogates immunosuppressive activity of myeloid-derived suppressor cells from prostate cancer patients. *Clin Cancer Res.* (2015) 21:3771–82. doi: 10.1158/1078-0432.ccr-14-3145
55. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer* (2013) 13:739–52. doi: 10.1038/nrc3581
56. Wang D, Sun H, Wei J, Cen B, DuBois RN. CXCL1 is critical for premetastatic niche formation and metastasis in colorectal cancer. *Cancer Res*. (2017) 77:3655–65. doi: 10.1158/0008-5472.can-16-3199
57. Grunwald B, Harant V, Schaten S, Fruhschutz M, Spallek R, Hochst B, et al. Pancreatic premalignant lesions secrete tissue inhibitor of metalloproteinases-1, which activates hepatic stellate cells via CD63 signaling to create a premetastatic niche in the liver. *Gastroenterology* (2016) 151:1011–24 e7. doi: 10.1053/j.gastro.2016.07.043
58. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and

human myeloid-derived suppressor cells. *J Clin Invest*. (2010) 120:457–71. doi: 10.1172/jci40483
59. Deguchi A, Tomita T, Ohto U, Takemura K, Kitao A, Akashi-Takamura S, et al. Eritoran inhibits S100A8-mediated TLR4/MD-2 activation and tumor growth by changing the immune microenvironment. *Oncogene* (2016) 35:1445–56. doi: 10.1038/onc.2015.211
60. Wang Z, Xiong S, Mao Y, Chen M, Ma X, Zhou X, et al. Periostin promotes immunosuppressive premetastatic niche formation to facilitate breast tumour metastasis. *J Pathol*. (2016) 239:484–95. doi: 10.1002/path.4747
61. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol*. (2016) 1:aaf8943. doi: 10.1126/sciimmunol.aaf8943
62. Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, et al. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell* (2009) 15:35–44. doi: 10.1016/j.ccr.2008.11.012
63. Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M, et al. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci USA*. (2009) 106:6742–7. doi: 10.1073/pnas.0902280106
64. Papaspyridonos M, Matei I, Huang Y, do Rosario Andre M, Brazier-Mitouart H, Waite JC, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. *Nat Commun*. (2015) 6:6840. doi: 10.1038/ncomms7840
65. Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, et al. The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. *Nat Cell Biol*. (2008) 10:1349–55. doi: 10.1038/ncb1794.
66. Yang X, Lin Y, Shi Y, Li B, Liu W, Yin W, et al. FAP promotes immunosuppression by cancer-associated fibroblasts in the tumor microenvironment via STAT3-CCL2 signaling. *Cancer Res*. (2016) 76:4124–35. doi: 10.1158/0008-5472.can-15-2973
67. Fan Q, Gu D, Liu H, Yang L, Zhang X, Yoder MC, et al. Defective TGF-beta signaling in bone marrow-derived cells prevents hedgehog-induced skin tumors. *Cancer Res.* (2014) 74:471–83. doi: 10.1158/0008-5472.can-13-2134-t
68. Shi H, Zhang J, Han X, Li H, Xie M, Sun Y, et al. Recruited monocytic myeloid-derived suppressor cells promote the arrest of tumor cells in the premetastatic niche through an IL-1beta-mediated increase in E-selectin expression. *Int J Cancer* (2017) 140:1370–83. doi: 10.1002/ijc.30538
69. Kitamura T, Fujishita T, Loetscher P, Revesz L, Hashida H, Kizaka-Kondoh S, et al. Inactivation of chemokine (C-C motif) receptor 1 (CCR1) suppresses colon cancer liver metastasis by blocking accumulation of immature myeloid cells in a mouse model. *Proc Natl Acad Sci USA*. (2010) 107:13063–8. doi: 10.1073/pnas.1002372107
70. Yan HH, Jiang J, Pang Y, Achyut BR, Lizardo M, Liang X, et al. CCL9 induced by TGFbeta signaling in myeloid cells enhances tumor cell survival in the premetastatic organ. *Cancer Res*. (2015) 75:5283–98. doi: 10.1158/0008-5472.can-15-2282-t
71. Inamoto S, Itatani Y, Yamamoto T, Minamiguchi S, Hirai H, Iwamoto M, et al. Loss of SMAD4 promotes colorectal cancer progression by accumulation of myeloid-derived suppressor cells through the CCL15-CCR1 chemokine axis. *Clin Cancer Res.* (2016) 22:492–501. doi: 10.1158/1078-0432.ccr-15-0726
72. Kaplan RN, Psaila B, Lyden D. Bone marrow cells in the 'pre-metastatic niche': within bone and beyond. *Cancer Metastasis Rev*. (2006) 25:521–9. doi: 10.1007/s10555-006-9036-9
73. Schlesinger M, Bendas G. Contribution of very late antigen-4 (VLA-4) integrin to cancer progression and metastasis. *Cancer Metastasis Rev*. (2015) 34:575–91. doi: 10.1007/s10555-014-9545-x
74. Resheq YJ, Menzner AK, Bosch J, Tickle J, Li KK, Wilhelm A, et al. Impaired transmigration of myeloid-derived suppressor cells across human sinusoidal endothelium is associated with decreased expression of CD13. *J Immunol*. (2017) 199:1672–81. doi: 10.4049/jimmunol.1600466
75. Sangaletti S, Chiodoni C, Tripodo C, Colombo MP. Common extracellular matrix regulation of myeloid cell activity in the bone marrow and tumor microenvironments. *Cancer Immunol Immunother*. (2017) 66:1059–67. doi: 10.1007/s00262-017-2014-y
76. Cheng P, Corzo CA, Luetteke N, Yu B, Nagaraj S, Bui MM, et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J Exp Med*. (2008) 205:2235–49. doi: 10.1084/jem.20080132
77. Sinha P, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J Immunol.* (2008) 181:4666–75. doi: 10.4049/jimmunol.181.7.4666
78. Maruhashi T, Kii I, Saito M, Kudo A. Interaction between periostin and BMP-1 promotes proteolytic activation of lysyl oxidase. *J Biol Chem*. (2010) 285:13294–303. doi: 10.1074/jbc.M109.088864
79. Nan J, Xing YF, Hu B, Tang JX, Dong HM, He YM, et al. Endoplasmic reticulum stress induced LOX-1(+) CD15(+) polymorphonuclear myeloid-derived suppressor cells in hepatocellular carcinoma. *Immunology* (2018) 154:144–55. doi: 10.1111/imm.12876
80. Gupta GP, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, et al. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* (2007) 446:765–70. doi: 10.1038/nature05760
81. Huang X, Jia L, Qian Z, Jia Y, Chen X, Xu X, et al. Diversity in human placental microvascular endothelial cells and macrovascular endothelial cells. *Cytokine* (2018) 111:287–94. doi: 10.1016/j.cyto.2018.09.009
82. Jean C, Chen XL, Nam JO, Tancioni I, Uryu S, Lawson C, et al. Inhibition of endothelial FAK activity prevents tumor metastasis by enhancing barrier function. *J Cell Biol.* (2014) 204:247–63. doi: 10.1083/jcb.201307067
83. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature* (2018) 554:538–43. doi: 10.1038/nature25492
84. Bellomo C, Caja L, Moustakas A. Transforming growth factor beta as regulator of cancer stemness and metastasis. *Br J Cancer* (2016) 115:761–9. doi: 10.1038/bjc.2016.255
85. Shvedova AA, Kisin ER, Yanamala N, Tkach AV, Gutkin DW, Star A, et al. MDSC and TGFbeta are required for facilitation of tumor growth in the lungs of mice exposed to carbon nanotubes. *Cancer Res*. (2015) 75:1615–23. doi: 10.1158/0008-5472.can-14-2376
86. Lebrun A, Lo Re S, Chantry M, Izquierdo Carerra X, Uwambayinema F, Ricci D, et al. CCR2(+) monocytic myeloid-derived suppressor cells (M-MDSCs) inhibit collagen degradation and promote lung fibrosis by producing transforming growth factor-beta1. *J Pathol*. (2017) 243:320–30. doi: 10.1002/path.4956
87. Hochst B, Schildberg FA, Sauerborn P, Gabel YA, Gevensleben H, Goltz D, et al. Activated human hepatic stellate cells induce myeloid derived suppressor cells from peripheral blood monocytes in a CD44-dependent fashion. *J Hepatol*. (2013) 59:528–35. doi: 10.1016/j.jhep.2013.04.033
88. Li J, Wang L, Chen X, Li L, Li Y, Ping Y, et al. CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-beta-mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncoimmunology* (2017) 6:e1320011. doi: 10.1080/2162402x.2017.1320011
89. Huang Y, Song N, Ding Y, Yuan S, Li X, Cai H, et al. Pulmonary vascular destabilization in the premetastatic phase facilitates lung metastasis. *Cancer Res.* (2009) 69:7529–37. doi: 10.1158/0008-5472.can-08-4382
90. Hiratsuka S, Goel S, Kamoun WS, Maru Y, Fukumura D, Duda DG, et al. Endothelial focal adhesion kinase mediates cancer cell homing to discrete regions of the lungs via E-selectin up-regulation. *Proc Natl Acad Sci USA*. (2011) 108:3725–30. doi: 10.1073/pnas.1100446108
91. Bordeleau F, Mason BN, Lollis EM, Mazzola M, Zanotelli MR, Somasegar S, et al. Matrix stiffening promotes a tumor vasculature phenotype. *Proc Natl Acad Sci USA*. (2017) 114:492–97. doi: 10.1073/pnas.1613855114
92. Noma K, Smalley KS, Lioni M, Naomoto Y, Tanaka N, El-Deiry W, et al. The essential role of fibroblasts in esophageal squamous cell carcinoma-induced angiogenesis. *Gastroenterology* (2008) 134:1981–93. doi: 10.1053/j.gastro.2008.02.061
93. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, et al. Functions of S100 proteins. *Curr Mol Med*. (2013) 13:24–57. doi: 10.2174/1566524011307010024
94. Ostrand-Rosenberg S. Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. *Cancer Immunol Immunother*. (2010) 59:1593–600. doi: 10.1007/s00262-010-0855-8

95. Liu Z, Wang J, Xing W, Peng Y, Quan J, Fan X. LPS binding to HMGB1 promotes angiogenic behavior of endothelial cells through inhibition of p120 and CD31 via ERK/P38/Src signaling. *Eur J Cell Biol*. (2017) 96:695–704. doi: 10.1016/j.ejcb.2017.07.004
96. Burke M, Choksawangkarn W, Edwards N, Ostrand-Rosenberg S, Fenselau C. Exosomes from myeloid-derived suppressor cells carry biologically active proteins. *J Proteome Res*. (2014) 13:836–43. doi: 10.1021/pr400879c
97. Yao H, Price TT, Cantelli G, Ngo B, Warner MJ, Olivere L, et al. Leukaemia hijacks a neural mechanism to invade the central nervous system. *Nature* (2018) 560:55–60. doi: 10.1038/s41586-018-0342-5
98. Spampinato SF, Merlo S, Sano Y, Kanda T, Sortino MA. Astrocytes contribute to Abeta-induced blood-brain barrier damage through activation of endothelial MMP9. *J Neurochem*. (2017) 142:464–77. doi: 10.1111/jnc.14068
99. Kim GS, Yang L, Zhang G, Zhao H, Selim M, McCullough LD, et al. Critical role of sphingosine-1-phosphate receptor-2 in the disruption of cerebrovascular integrity in experimental stroke. *Nat Commun*. (2015) 6:7893. doi: 10.1038/ncomms8893
100. Ebrahem Q, Chaurasia SS, Vasanji A, Qi JH, Klenotic PA, Cutler A, et al. Cross-talk between vascular endothelial growth factor and matrix metalloproteinases in the induction of neovascularization *in vivo*. *Am J Pathol*. (2010) 176:496–503. doi: 10.2353/ajpath.2010.080642
101. Lobb RJ, Lima LG, Moller A. Exosomes: Key mediators of metastasis and pre-metastatic niche formation. *Semin Cell Dev Biol*. (2017) 67:3–10. doi: 10.1016/j.semcdb.2017.01.004
102. Ahn GO, Brown JM. Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. *Cancer Cell* (2008) 13:193–205. doi: 10.1016/j.ccr.2007.11.032
103. Tjiu JW, Chen JS, Shun CT, Lin SJ, Liao YH, Chu CY, et al. Tumor-associated macrophage-induced invasion and angiogenesis of human basal cell carcinoma cells by cyclooxygenase-2 induction. *J Invest Dermatol*. (2009) 129:1016–25. doi: 10.1038/jid.2008.310
104. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, et al. Tumor exosomal RNAs promote lung pre-metastatic niche formation by activating alveolar epithelial TLR3 to recruit neutrophils. *Cancer Cell* (2016) 30:243–56. doi: 10.1016/j.ccell.2016.06.021
105. Li K, Chen Y, Li A, Tan C, Liu X. Exosomes play roles in sequential processes of tumor metastasis. *Int J Cancer* (2018). doi: 10.1002/ijc.31774. [Epub ahead of print].
106. Zoller M. Janus-faced myeloid-derived suppressor cell exosomes for the good and the bad in cancer and autoimmune disease. *Front Immunol*. (2018) 9:137. doi: 10.3389/fimmu.2018.00137
107. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. *J Biol Chem*. (2013) 288:10849–59. doi: 10.1074/jbc.M112.446831
108. Noman MZ, Janji B, Hu S, Wu JC, Martelli F, Bronte V, et al. Tumor-promoting effects of myeloid-derived suppressor cells are potentiated by hypoxia-induced expression of miR-210. *Cancer Res*. (2015) 75:3771–87. doi: 10.1158/0008-5472.can-15-0405
109. Walsh JE, Clark AM, Day TA, Gillespie MB, Young MR. Use of alpha,25-dihydroxyvitamin D3 treatment to stimulate immune infiltration into head and neck squamous cell carcinoma. *Hum Immunol*. (2010) 71:659–65. doi: 10.1016/j.humimm.2010.04.008
110. Kusmartsev S, Cheng F, Yu B, Nefedova Y, Sotomayor E, Lush R, et al. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. *Cancer Res* (2003) 63:4441–9.
111. Kusmartsev S, Su Z, Heiser A, Dannull J, Eruslanov E, Kubler H, et al. Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. *Clin Cancer Res*. (2008) 14:8270–8. doi: 10.1158/1078-0432.ccr-08-0165
112. Bruns H, Bottcher M, Qorraj M, Fabri M, Jitschin S, Dindorf J, et al. CLL-cell-mediated MDSC induction by exosomal miR-155 transfer is disrupted by vitamin D. *Leukemia* (2017) 31:985–88. doi: 10.1038/leu.2016.378
113. Veltman JD, Lambers ME, van Nimwegen M, Hendriks RW, Hoogsteden HC, Hegmans JP, et al. Zoledronic acid impairs myeloid differentiation to tumour-associated macrophages in mesothelioma. *Br J Cancer* (2010) 103:629–41. doi: 10.1038/sj.bjc.6605814
114. Zhuang J, Zhang J, Lwin ST, Edwards JR, Edwards CM, Mundy GR, et al. Osteoclasts in multiple myeloma are derived from Gr-1+CD11b+myeloid-derived suppressor cells. *PLoS ONE* (2012) 7:e48871. doi: 10.1371/journal.pone.0048871
115. Porembka MR, Mitchem JB, Belt BA, Hsieh CS, Lee HM, Herndon J, et al. Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth. *Cancer Immunol Immunother*. (2012) 61:1373–85. doi: 10.1007/s00262-011-1178-0
116. Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. *Cancer Res*. (2007) 67:11438–46. doi: 10.1158/0008-5472.can-07-1882
117. Priceman SJ, Sung JL, Shaposhnik Z, Burton JB, Torres-Collado AX, Moughon DL, et al. Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. *Blood* (2010) 115:1461–71. doi: 10.1182/blood-2009-08-237412
118. Zhang Y, Liu Q, Zhang M, Yu Y, Liu X, Cao X. Fas signal promotes lung cancer growth by recruiting myeloid-derived suppressor cells via cancer cell-derived PGE2. *J Immunol*. (2009) 182:3801–8. doi: 10.4049/jimmunol.0801548
119. Hurez V, Daniel BJ, Sun L, Liu AJ, Ludwig SM, Kious MJ, et al. Mitigating age-related immune dysfunction heightens the efficacy of tumor immunotherapy in aged mice. *Cancer Res*. (2012) 72:2089–99. doi: 10.1158/0008-5472.can-11-3019
120. Condamine T, Kumar V, Ramachandran IR, Youn JI, Celis E, Finnberg N, et al. ER stress regulates myeloid-derived suppressor cell fate through TRAIL-R-mediated apoptosis. *J Clin Invest*. (2014) 124:2626–39. doi: 10.1172/jci74056
121. Dominguez GA, Condamine T, Mony S, Hashimoto A, Wang F, Liu Q, et al. Selective targeting of myeloid-derived suppressor cells in cancer patients using DS-8273a, an agonistic TRAIL-R2 antibody. *Clin Cancer Res*. (2017) 23:2942–50. doi: 10.1158/1078-0432.ccr-16-1784
122. Eksioglu EA, Chen X, Heider KH, Rueter B, McGraw KL, Basiorka AA, et al. Novel therapeutic approach to improve hematopoiesis in low risk MDS by targeting MDSCs with the Fc-engineered CD33 antibody BI 836858. *Leukemia* (2017) 31:2172–80. doi: 10.1038/leu.2017.21
123. Garton AJ, Seibel S, Lopresti-Morrow L, Crew L, Janson N, Mandiyan S, et al. Anti-KIT monoclonal antibody treatment enhances the antitumor activity of immune checkpoint inhibitors by reversing tumor-induced immunosuppression. *Mol Cancer Ther*. (2017) 16:671–80. doi: 10.1158/1535-7163.mct-16-0676
124. Cappello P, Tonoli E, Curto R, Giordano D, Giovarelli M, Novelli F. Anti-alpha-enolase antibody limits the invasion of myeloid-derived suppressor cells and attenuates their restraining effector T cell response. *Oncoimmunology* (2016) 5:e1112940. doi: 10.1080/2162402x.2015.1112940
125. Kobayashi M, Chung JS, Beg M, Arriaga Y, Verma U, Courtney K, et al. Blocking monocytic myeloid-derived suppressor cell function via anti-DC-HIL/GPNMB antibody restores the *in vitro* integrity of t cells from cancer patients. *Clin Cancer Res*. (2018) 25:828–38. doi: 10.1158/1078-0432.ccr-18-0330
126. Gajos-Michniewicz A, Duechler M, Czyz M. MiRNA in melanoma-derived exosomes. *Cancer Lett*. (2014) 347:29–37. doi: 10.1016/j.canlet.2014.02.004
127. Wang Y, Tian J, Tang X, Rui K, Tian X, Ma J, et al. Exosomes released by granulocytic myeloid-derived suppressor cells attenuate DSS-induced colitis in mice. *Oncotarget* (2016) 7:15356–68. doi: 10.18632/oncotarget.7324
128. Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, et al. Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of midkine. *Nature* (2017) 546:676–80. doi: 10.1038/nature22977
129. Cox TR, Rumney RMH, Schoof EM, Perryman L, Hoye AM, Agrawal A, et al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* (2015) 522:106–10. doi: 10.1038/nature14492
130. Ugel S, Peranzoni E, Desantis G, Chioda M, Walter S, Weinschenk T, et al. Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. *Cell Rep*. (2012) 2:628–39. doi: 10.1016/j.celrep.2012.08.006
131. Anani W, Shurin MR. Targeting myeloid-derived suppressor cells in cancer. *Adv Exp Med Biol*. (2017) 1036:105–28. doi: 10.1007/978-3-319-67577-0_8

132. Najjar YG, Finke JH. Clinical perspectives on targeting of myeloid derived suppressor cells in the treatment of cancer. *Front Oncol.* (2013) 3:49. doi: 10.3389/fonc.2013.00049
133. Kulbersh JS, Day TA, Gillespie MB, Young MR. 1alpha,25-Dihydroxyvitamin D(3) to skew intratumoral levels of immune inhibitory CD34(+) progenitor cells into dendritic cells. *Otolaryngol Head Neck Surg.* (2009) 140:235–40. doi: 10.1016/j.otohns.2008.11.011
134. Zhang H, Maric I, DiPrima MJ, Khan J, Orentas RJ, Kaplan RN, et al. Fibrocytes represent a novel MDSC subset circulating in patients with metastatic cancer. *Blood* (2013) 122:1105–13. doi: 10.1182/blood-2012-08-449413
135. Hengesbach LM, Hoag KA. Physiological concentrations of retinoic acid favor myeloid dendritic cell development over granulocyte development in cultures of bone marrow cells from mice. *J Nutr.* (2004) 134:2653–9. doi: 10.1093/jn/134.10.2653
136. Li L, Zhang J, Diao W, Wang D, Wei Y, Zhang CY, et al. MicroRNA-155 and MicroRNA-21 promote the expansion of functional myeloid-derived suppressor cells. *J Immunol.* (2014) 192:1034–43. doi: 10.4049/jimmunol.1301309
137. Martire-Greco D, Rodriguez-Rodrigues N, Castillo LA, Vecchione MB, de Campos-Nebel M, Cordoba Moreno M, et al. Novel use of all-trans-retinoic acid in a model of lipopolysaccharide-immunosuppression to decrease the generation of myeloid-derived suppressor cells by reducing the proliferation of CD34+ precursor cells. *Shock* (2017) 48:94–103. doi: 10.1097/shk.0000000000000812

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REVIEW
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Myeloid Derived Suppressor Cells Interactions With Natural Killer Cells and Pro-angiogenic Activities: Roles in Tumor Progression

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Myeloid-derived suppressor cells (MDSCs) contribute to the induction of an immune suppressive/anergic, tumor permissive environment. MDSCs act as immunosuppression orchestrators also by interacting with several components of both innate and adaptive immunity. Natural killer (NK) cells are innate lymphoid cells functioning as primary effector of immunity, against tumors and virus-infected cells. Apart from the previously described anergy and hypo-functionality of NK cells in different tumors, NK cells in cancer patients show pro-angiogenic phenotype and functions, similar to decidual NK cells. We termed the pro-angiogenic NK cells in the tumor microenvironment "tumor infiltrating NK" (TINKs), and peripheral blood NK cells in cancer patients "tumor associated NK" (TANKs). The contribution of MDSCs in regulating NK cell functions in tumor-bearing host, still represent a poorly explored topic, and even less is known on NK cell regulation of MDSCs. Here, we review whether the crosstalk between MDSCs and NK cells can impact on tumor onset, angiogenesis and progression, focusing on key cellular and molecular interactions. We also propose that the similarity of the properties of tumor associated/tumor infiltrating NK and MDSC with those of decidual NK and decidual MDSCs during pregnancy could hint to a possible onco-fetal origin of these pro-angiogenic leukocytes.

Keywords: myeloid derived suppressor cell (MDSC), natural killer cells (NK cells), angiogenesis, cytokines, tumor microenvironment, decidua

INTRODUCTION

The tumor microenvironment (TME) shapes the fate of tumor onset and progression, by regulating cell growth, invasiveness, immune escape, dissemination and clinical outcome (1, 2). It is now clear that the contribution of tissue-resident immune cells in supporting or limiting tumor growth, metastasis and resistance to therapies has a master role (2, 3). The immune cell effector's capabilities of reaching, recognizing, and eliminating the tumor targets is conditioned by other microenvironment cells and determinants, turning the immune system from early strategic line of defense, into a pro-tumor weapon (1–3). TME employs multiple mechanisms to switch off the anti-tumor functions of immune cells: it can destabilize and polarize the innate cell compartment (macrophages, neutrophils and dendritic cells as well as innate lymphoid cells), the adaptive

immunity (T and B lymphocytes), stromal cells (cancer-associated fibroblasts) or endothelial cells (tumor associated capillary or lymphatic vessels) to favor growth and dissemination (1–4). Plasticity of immune cells, referred as the ability of immune cells to be differentially polarized (for example, acquisition of different or opposite phenotypes and functions) within different (micro/macro) environments (1, 2, 4–6) can represent a friend or a foe. Among the most interesting players in the TME regulation of cancer and metastases are myeloid-derived suppressor cells (MDSCs). MDSCs can directly or indirectly (by interacting with several components of both innate and adaptive immunity) contribute to the induction of an immune suppressive environment (7, 8), and angiogenesis (4, 9–11). We will discuss their crosstalk with Natural killer (NK) cells.

NK cells are innate lymphoid cells (ILC) and act as primary effectors of innate immunity, against tumors and virus-infected cells (12). In cancer, NK cells show anergy and hypo-functionality (13–15). NK cells in different tumors have been described by us (4, 16, 17) and other groups (18, 19) to acquire pro-angiogenic phenotype and pro-tumor functions.

MDSCs are recruited and expanded in the TME, in different types of mouse and human cancers (20–24). MDSCs can restrain the $CD8^+$ cytotoxic T and NK cells, both of which are anti-cancer, directly influencing the pro-tumor TME. In this review, we will address MDSC-associated angiogenesis and the crosstalk between MDSCs and NK cells, an under-investigated field, and we will focus on relevant cellular and molecular events orchestrating NK-MDSC interactions within the TME, which can impact on tumor insurgence, progression, and angiogenesis.

NK CELL PHENOTYPE AND FUNCTIONS IN CANCER

NK cells are cytolytic and cytokine-producing effector innate lymphoid cells (ILC), representing a first line of defense against virally-infected and transformed cells (12). Spits et al. assigned NK cells as a prototypical ILC family member and classified NK cells as ILC1 subtype, as a consequence of their ability to produce IFNγ, following T-bet and EOMES expression from the $ID2^+$ ILC precursor (25). Recently, Vivier et al. put forward that NK cells originate from a separate cell lineage from ILC1. NK cells and ILC1 share the ability to produce IFNγ, following T-bet expression (26). NK cells and ILC1, however, are functionally different: while NK cells are strongly cytotoxic and release perforin, ILC1s cannot release perforin (26).

The field of NK cell biology has expanded well beyond their cytotoxic functions, underlying new roles related to the vast array of cytokines produced by these cells. NK cells are now known to act in immune responses against bacterial (27) and fungal (28, 29) organisms. They have also been shown to play a role in both bone marrow rejection and bone marrow cell engraftment (30). Further NK cell immune regulatory (31) and tissue-regenerative properties (32) have been discovered in viral resistance models.

NK cell cytolytic functions are exerted by perforin and granzyme production and cytokine release. These properties are regulated by a balance between signals from inhibitory receptors (killer Ig-like receptors [KIRs] and the heterodimeric C-type lectin receptor [NKG2A]) as well as activating receptors (the NCRs: NKp46, NKp30, NKp44, and the C-type lectin-like activating immunoreceptor NKG2D), recognizing specific ligands on their cellular targets (12). Peripheral NK cells are predominantly (from 90 to 95%) $CD56^{dim}CD16^+$ cytotoxic NK cells, that exert their effector functions by perforin/granzyme release and antibody dependent cellular cytotoxicity (ADCC). A minor NK subset, within total circulating NK cells (5–10%), exhibits the $CD56^{bright}CD16^-$ phenotype and is able to produce high and constant levels of anti-tumor cytokines, such as IFNγ and TNFα (12), $CD56^{bright}CD16^-$ NK cells are abundant in healthy and neoplastic solid tissues (33).

Pro-angiogenic NK cells have been found in wound healing models (17), a pro-angiogenic NK subset has been also characterized within the developing decidua: decidual (or uterine) NK cells (dNK), that will be discussed later in this review.

Anergic NK cells have been characterized in several tumors, where local immunosuppression resulted in NK cells downregulating NKG2D surface antigen expression, impaired degranulation capabilities, limited abilities to release perforin, granzyme and anti-tumor cytokines (34–38).

We were the first in demonstrating that NK cells in cancer patients (non-small cell lung cancer, NSCLC) (17, 39) colorectal cancer (40) and in malignant pleural effusions (16), show a pro-angiogenic phenotype and function, identified as $CD56^{bright}CD16^-VEGF^{high}CXCL8^+IFN^{low}$ and share several features with the highly pro-angiogenic dNK cells (17, 39, 40). In cancer patients, NK cells mimic behavior of decidual NK, they exhibit a dNK-like phenotype, release pro-angiogenic and pro-metastatic factors and functionally support angiogenesis (4, 16–19, 36, 39, 40). We termed the pro-angiogenic NK cells that are in the TME: "tumor infiltrating NK" (TINKs) and peripheral blood pro-angiogenic NK cells in cancer patients "tumor associated NK" (TANKs) (17).

MYELOID-DERIVED SUPPRESSOR CELL PHENOTYPE AND FUNCTIONS IN CANCER

MDSCs identify a heterogeneous immature and mature cell population generated from common hematopoietic progenitor cell. Two major MDSC subsets have been characterized based on their ability a) to phenotypically resemble polymorphonuclear (PMN) cells, termed PMN-MDSCs b) to resemble monocytes, defined M-MDSCs, for their surface markers. Both cell subsets are endowed with potent inhibitory functions against $CD8^+$ cytotoxic T cells and NK cells, thus inducing a tolerogenic state and acquiring pro-angiogenic properties (23). In mice, PMN-MDSCs are characterized by $CD11b^+Ly6G^+Ly6C^{lo}$ while M-MDSCs by $CD11b^+Ly6G^-Ly6C^{hi}$ surface markers. In humans, PMN-MDSCs are identified as $CD11b^+CD14^-CD15^+$ or $CD11b^+CD14^-CD66b^+$, and M-MDSCs as $CD11b^+CD14^+HLA\text{-}DR^{-/lo}CD15^-$ (20, 41). LOX 1 (Lectin type oxidized receptor-1) represents a more recent marker that has been identified on human PMN-MDSCs, however further confirmation is needed (42).

The immature phenotype of MDSCs is related to the constitutive activation of signal transducer and activator of transcription (STAT)-3, that interferes with the completion of functional cell maturation. The expansion of this subset in tumor patients and tumor-bearing mice is driven also by different factors, such as IRF8, C/EBPβ, Notch, adenosine receptors A2b signaling, and NLRP3 (43). For their immunoregulatory function, the MDSCs requires different pro-inflammatory stimuli, like CSF3, IL-1β, IL-6, and prostaglandin E2 (PGE2), through activation of the NF-κB pathway, as well as of STAT1, STAT6, and cyclooxygenase 2 (COX2) signaling (43), as described more in depth later. Immunosuppressive functions exerted by MDSCs are also mediated through the inducible form of nitric oxide synthase (NOS2) that produces nitric oxide (NO), arginase 1 (ARG1), TGFβ, IL-10, COX2, and indoleamine 2,3-dioxygenase (IDO) (44). PGE2 and HMGB1 are also involved in immune suppression (43). In cancer patients, MDSC expansion in the peripheral blood is correlated with poor clinical outcome and with advanced clinical stage (45–47). Tumors growing in mice lead to the expansion and activation of myeloid cells (48, 49) with similar activities than the human counterparts, resulting in impairment of anti-tumor T cell responses (50).

It has been shown that MDSCs are able to favor the conversion of naive $CD4^+$ T cells into Tregs. Retinoids and MDSC-derived TGFβ can promote the trans-differentiation of Th17 cells into $Foxp3^+$ Tregs (51).

MDSC AND NK CELL CROSSTALK

Immunosuppressive activities by MDSCs have been largely described to be directed toward T cells. Emerging evidence suggests that MDSCs can also interact and regulate the function of other immune cells, including macrophages, DCs and NK cells (7, 8, 52–54). The contribution of MDSCs in regulating NK cell function in tumor-bearing host, still represent a poorly explored topic. MDSCs produce TGFβ which we and others have shown to be a master regulator of NK cell functions in tumors (4, 13, 17, 39, 55–58) (**Figure 1**). Studies in the literature showed that co-culture of MDSCs with NK cells resulted in impaired tumor cell cytotoxic activity by NK cells and induction of immunotolerance (59, 60). These alterations derived both by MDSC/NK direct interaction (e.g., PDL-1 checkpoint ligand expression and reactive oxygen species production) and via soluble factors (described later in the manuscript). MDSCs have been observed to reduce NK cells tumor suppressive activity (52), and chronic inflammation increases these effects. Several pro-inflammatory cytokines have been reported to orchestrate MDSC/NK crosstalk. Large number of $CD11b^+Gr\text{-}1^+$ cells have been found to accumulate in the spleen of tumor-bearing mice and, when adoptively transferred both into tumor-bearing and naïve mice, were able to inhibit NK cell cytotoxicity, by limiting the NK ability to produce perforin *in vivo* and *in vitro* (53). MDSC-mediated NK cell anergy has been associated with the ability of MDSCs to downregulate CD247 expression on the NK cell surface (61). CD247 is a key subunit of natural cytotoxicity receptors (NCRs) NKp46, NKp30, and Fcγ RIII (CD16) (61). MDSCs can inhibit NK cell function by interacting with the NKp30 receptor (62). MDSC/NK cells co-culture results in down-regulation of NKG2D, impaired degranulation capabilities and decreased secretion of IFNγ by NK cells (63). The interaction between MDSCs $CD11b^+Ly6C^{med}Ly6G^+$ and NK cells ($CD3^-NK1.1^+$) in the murine pre-metastatic niche has been reported to be critical for metastases establishment (64). The cytotoxicity of NK cells in breast cancer is significantly decreased in the presence of MDSCs, resulting in increased metastatic potential (64). MDSCs inhibit the anti-tumor reactivity of NK cells, promote angiogenesis (65), establish pre-metastatic niches (66), and recruit other immunosuppressive cells (67). MDSC accumulation has been demonstrated to occur, following surgery both in human and mice, which results in dysfunctional NK cells (68–70).

The immune suppressive TME leads to phenotype and functional alterations of several players, including NK cells and MDSCs. Most of soluble molecules within the TME include factors able in shaping NK cell and MDSC response and several of them are shared interactors regulating MDSC/NK crosstalk. Here, we discussed selected soluble factors modulating MDSC/NK cell crosstalk within the TME, as potential candidates to target aberrant phenotype/function endowed with pro-tumor and pro-angiogenic activities.

CYTOKINES AND OTHER MEDIATORS IN NK AND MDSC REGULATION

The STAT family are transcription factors that are activated in response to growth factors and cytokines and mediate downstream signaling (71–74). STATs are dysregulated in a broad range of cancer types. STATs have been shown to play diverse roles in innate and adaptive immune cells in the TME (75–77). While STAT2 and STAT4 promote the anti-tumor immune response, STAT3 and STAT6 mediate immunosuppression in the TME, and STAT1 and STAT5 have been implicated in both activation and suppression of the anti-tumor immune response (78). STAT3 activation in an immature MDSC subset, has been found to be crucial for NF-κB activation, resulting in enhanced release of IDO, that limit NK cell proliferation, activation and effector functions (79) (**Figure 2**). Several studies demonstrated a link between STAT3 blockade, TGFβ inhibition and increased tumor surveillance by NK cells (80, 81). Peripheral and tumor-associated NK cells from STAT3-targeted tumor-bearing mice expressed elevated levels of NK activation markers NKG2D, CD69, Fas ligand (FasL) granzyme B, perforin, and IFNγ, resulting in reduced tumor growth and enhanced survival (80, 81).

IL-2 induced activation of STAT5 leads to NK cell production of perforin, granzyme and IFNγ (82). JAK3-mediated activation of the transcription factor STAT5 is critical in IL-2–stimulated NK cells *in vitro* and Jak3 inhibition has been found in NK cells co-cultured with MDSC isolated from the spleen of tumor-bearing mice associated with reduced STAT5 in NK cells (62). STAT3/STAT5 activation was observed by us in TANKs from colon cancer patients (40). We have shown that treatment with

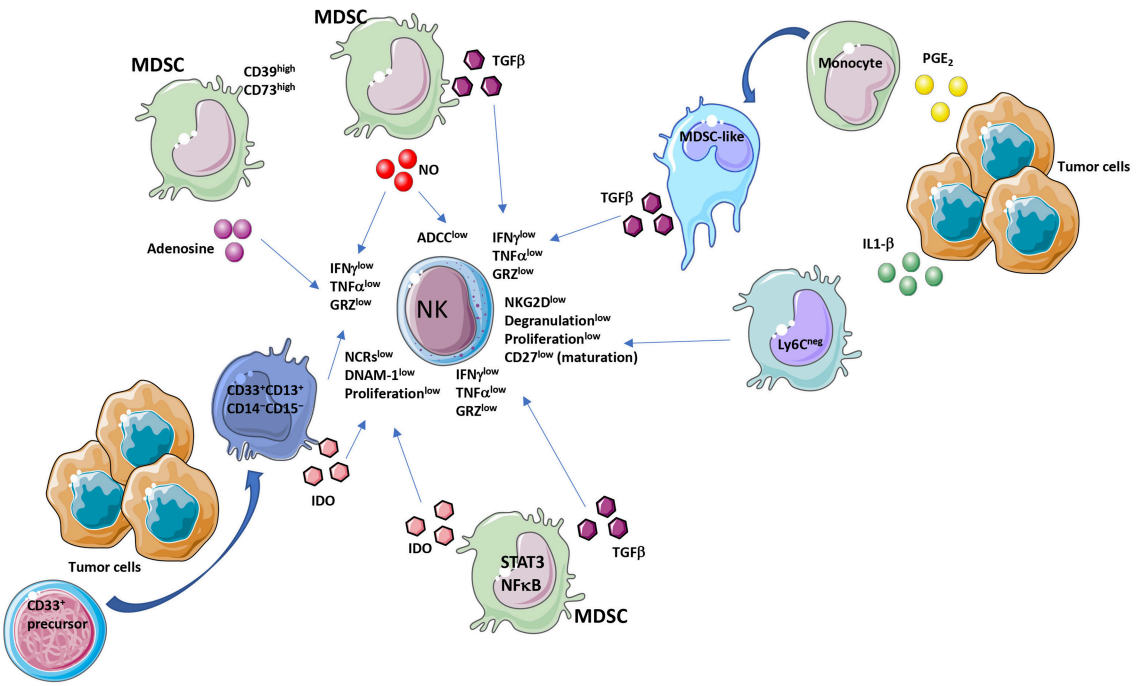

FIGURE 1 | MDSC and NK crosstalk within the tumor microenvironment (TME). Immunosuppressive activities of MDSCs on NK cells act by diverse molecular and cellular mediators. MDSC affect NK cell functionality by several major released factors, among which TGFβ. TGFβ is produced by MDSC or by MDSC-like cells, originated from PGE2 exposed monocytes. Another mediator is IDO produced directly from MDSCs or from a $CD33^{+}CD13^{+}CD14^{-}CD15^{-}$ subset, derived from $CD33^{+}$ precursors. Adenosine from $CD39^{high}CD73^{high}$ MDSCs is a further major NK suppressive factor. MDSC effectors decrease NKG2D, NCRs, IFNγ, TNFα, perforin, granzyme levels and ADCC in NK cells.

pimozide, a STAT5 inhibitor, reduced endothelial cell capability to form capillary-like networks, inhibiting VEGF and angiogenin production without affecting the levels of TIMP1, TIMP2, and MMP9, indicating that STAT5 is involved in cytokine modulation but not invasion-associated molecules in colon cancer TANKs (40).

MDSCs release TGFβ in the TME (23, 48, 83) (**Figure 1**). TGFβ exerts anti-tumorigenic effects at early stages, while during tumor progression it acts as crucial orchestrator of angiogenesis, induction of immunosuppression and metastases (84–86). In a murine model of liver cancer, tumor derived MDSCs have been reported to induce NK cell anergy, exhibited as reduced NKG2D expression, degranulation capability, cytotoxicity and IFNγ release *in vitro* and *in vivo*, through membrane-bound $TGFβ_1$ (59). Blocking of membrane-bound TGFβ on MDSCs was able to subvert the inhibitory effects on NK cells, demonstrating that MDSC/NK cell-cell contact is necessary to induce MDSC-mediated NK cell anergy.

Elkabets et al. have identified a novel subset of MDSC induced by IL-1β, which lack Ly6C expression (52) (**Figure 1**). This subset was present at low frequency in tumor-bearing mice in the absence of IL-1β-induced inflammation; while under inflammatory conditions $Ly6C^{neg}$ MDSC were predominant. $Ly6C^{neg}$ MDSC impaired NK cell development and functions *in vitro* and *in vivo* (52) by reduction of NKG2D activating receptor (**Figure 1**). Another recently identified NK check-point is the IL-1R8 receptor (also known as SIGIRR, or TIR8), which is expressed on human and murine NK cells (87). IL-33, an "alarmin" molecule released upon tissue stress or damage by endothelial and epithelial cells (88, 89), is an IL-1 family member which binds to the ST2 receptor, expressed on immune cells. In murine models, IL-33, depending on the TME, can recruit immune cells with pro-tumor effects, including MDSCs, TAMs, and Tregs, or it can prevent tumor development by stimulating activation and migration of NK and $CD8^{+}$ T cells (88, 89). In humans, IL-33 is associated with poor prognosis in glioma, breast and ovarian cancers, clear-cell renal and hepatocellular carcinoma, while it is correlated with good prognosis in colorectal cancer and lung adenocarcinoma (88, 89).

Nitric Oxide (NO) molecule is a multifunctional gaseous transmitter, playing a key role in inflammation. Paradoxical effects of NO have been documented in cancer, since its anti- or pro-tumor activities are finely tuned by timing, location, and concentration (90, 91). NO production has been largely demonstrated as a key mechanism in MDSC-mediated immunosuppression (90, 92) (**Figure 1**). Some studies showed that autocrine production of NO by NK cells results in positive effect on NK cell function, and that human NK cells can express endothelial nitric oxide synthase (eNOS) but not inducible nitric oxide synthase (iNOS) (93, 94). In contrast, Stiff et al. recently demonstrated that NO production by MDSCs limits NK cell cytotoxicity by impairing Fc receptor-mediated NK cell function,

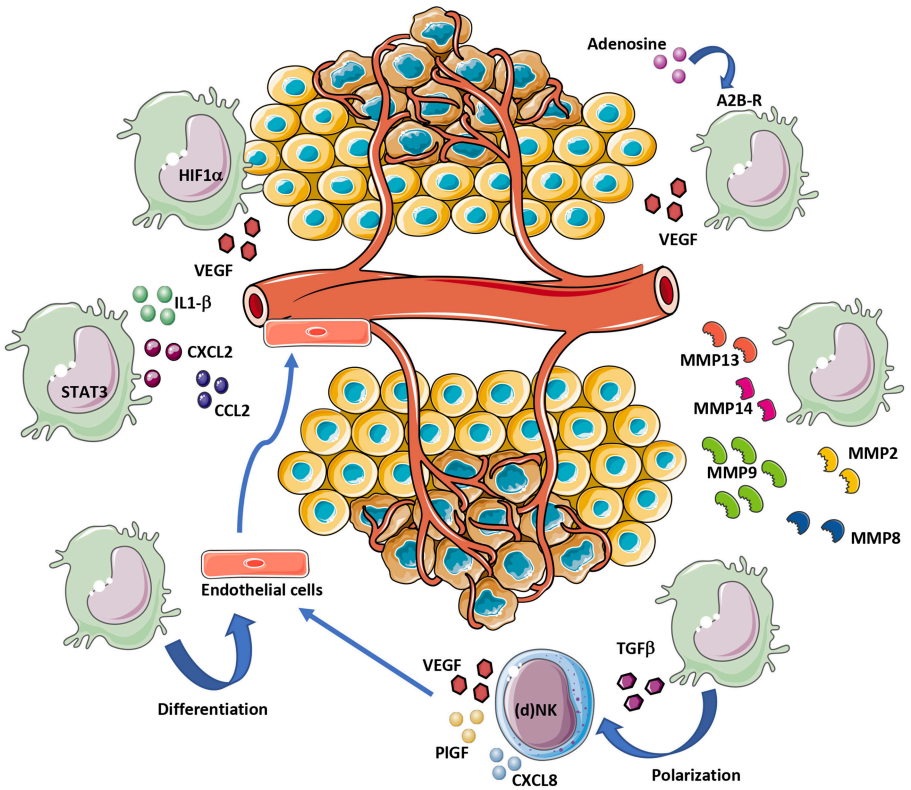

FIGURE 2 | MDSC contribution to tumor angiogenesis. MDSCs can support angiogenesis by different mechanisms. Hypoxia within the TME induce VEGF release directly from MDSCs or indirectly following exposure of MDSCs to TGFβ and adenosine. STAT3 activation in MDSCs also support angiogenesis, via IL1-β, CXCL2, and CCL2 secretion. MDSCs contribute to tumor angiogenesis by ECM remodeling via MMP-2/8/9/13/14 release. Finally, given their cell plasticity, MDSCs can transdifferentiate into endothelial-like cells.

resulting in altered ADCC (92). They also showed that co-culture of MDSCs with NK cells results in inhibited secretion of IFNγ and TNFα by NK cells (**Figure 2**).

Prostaglandin E_2 (PGE_2) is a prostanoid molecule generated by the COX2 inflammatory cascade that have been largely reported to be associated with pro-tumor activities, ranging from induction of tumor cell growth, enhancement of tumor cell migration, invasion, induction of immunosuppression and angiogenesis (95–97). Exposure of monocytes to PGE_2 results in the generation of a MDSC-like phenotype, together with induction of intracellular signaling pattern, which enables them to suppress NK cell anti-tumor activity in a TGFβ dependent manner (98) (**Figure 1**). The same effects were observed in NK cells co-culture with freshly isolated $CD14^+HLA\text{-}DR^{low/-}$ M-MDSC from patients with melanoma (98). Selective inhibition of COX limited the accumulation of $CD11b^+Gr1^+$ MDSCs in the spleen, providing improved *in vivo* clearance of NK-cell sensitive YAC-1 cells in murine 4T-1 tumor cells (98). In a mouse model of acute inflammation obtained using zymosan, infiltration of NK cells was an early event with production of IFNγ, which upregulated microsomal PGE synthase-1 (mPGES-1) and COX-1, resulting in sustained PGE_2 biosynthesis (99). PGE_2 inhibited lymphocyte function and generated myeloid-derived suppressor cells (99).

Indoleamine 2,3-dioxygenase (IDO) is an intracellular monomeric, heme-containing enzyme able to regulate the tryptophan catabolism into kynurenine (100, 101). Kynurenine production will result in inhibition of proliferation and effector functions in NK and T cells (78, 102–105). MDSCs have been reported as an IDO producer cells within the TME, in both humans and mice. An immature subset of MDSCs, characterized as $CD33^+CD13^+CD14^-CD15^-$, has been identified (79, 106). This subset has been found to be induced from $CD33^+$ precursor cells that, following co-culture with the human breast cancer cell line MDA-MB-23, result in elevated production of IDO (**Figure 1**). IDO synthesized by MDSCs blocked NK cell development, proliferation, and activation, resulting in dramatically decreased expression of NCR, NKG2D, and DNAM-1 and by reducing IFNγ release (107, 108) (**Figure 1**).

As a consequence of hypoxia and inflammation, high levels of adenosine, an immunosuppressive molecule, are released within the TME, (109). Adenosine acts by engaging four subtypes of P1 purinergic or adenosine receptors, A1, A2A, A2B, A3, A2AR, and A2BR, that have been found to be expressed in immune cells (109, 110). A1, A2A, A2B, A3, A2AR, and A2BR mRNA levels dramatically increase in inflammatory cells within the TME (110). Adenosine/adenosine receptor interactions result in subverted immune cell activities, leading to immunosuppression

and angiogenesis driven by inflammatory cells (109). The enzyme CD39 converts extracellular ATP to AMP, and CD73 converts AMP to adenosine. MDSCs are able to express high levels of CD39/CD73 in tumor lesions, resulting in higher secretion of adenosine (111, 112) (**Figure 2**). Adenosine inhibits NK cell anti-tumor activities by blocking granzyme exocytosis, impairing perforin and Fas ligand-mediated cytotoxic activity and limiting IFNγ/TNFα release (113) (**Figure 1**). $CD56^{bright}CD16^{-}$ NK cells produce adenosine through a CD38-mediated pathway, another mechanism to generate extracellular AMP (114). Finally, it has been demonstrated that adenosine signaling is involved in limiting NK cell maturation and that engagement of A2A adenosine receptor (A2AR) acts as a checkpoint in this process (115).

DECIDUAL NK AND MDSCs DURING PREGNANCY: A POSSIBLE ONCO-FETAL ORIGIN OF PRO-ANGIOGENIC LEUKOCYTES

During pregnancy, profound and complex changes occur in the female organism in order to regulate and control the immune response to the fetus, thus conferring tolerance from rejection. This level of regulation in maternal immune system is achieved through coordination and crosstalk of different immune cells, including NK cells, MDSCs, DCs, and Tregs. The dNK cells represent an NK cell subset that has been characterized within the developing decidua and constitutes approximately 70% of the lymphoid cells in the decidua (116, 117). dNK cells have a $CD56^{superbright}CD16^{-}VEGF^{high}PlGF^{high}$ phenotype (58, 116, 117) and are endowed with pro-angiogenic activities, necessary for spiral artery formation. dNK are associated with induction of a tolerogenic environment to host the fetus and permit the correct embryo implantation, both in humans and mice (116, 117). Low levels of dNK cells is associated with miscarriage (17, 116). We have described the expression of angiogenin, in NK from patients with colon cancer (40). Angiogenin was previously reported to be secreted by dNK (118, 119). The TANKs in patients with colon cancer also express MMP2, MMP9, and TIMP, as shared features with dNK cells (116, 120, 121) which could be relevant to the invasive capabilities and proangiogenic functions of colorectal cancer-NK cells (40). Maternal dNK KIR and HLA-C interaction has an effect on birth weight (122), particularly the paternal HLA-C, and correlates with pre-eclampsia and fetal growth restriction (123, 124).

In healthy pregnant women, significant increase in numbers of PMN-MDSCs are detected as compared to non-pregnant controls (125). The raise of PMN-MDSCs mainly occurs in the first trimester (126). Accordingly, reduced PMN-MDSCs are associated with miscarriage (126). The mechanisms involved in this regulation could be related to the release of ARG1, NO, IDO, and indirectly by recruitment and activation of dNK cells and Tregs (127, 128). Serum levels of ARG1, an important effector molecule for PMN-MDSC are significantly reduced in pre-eclampsia patients as compared to healthy pregnant women (129). Behavior of immune cells in tumors might resemble the one in the decidua (4).

MDSC AND TUMOR ANGIOGENESIS

MDSCs promote tumor progression also through non-immune activities, by stimulating pre-metastatic niche formation, invasion (130, 131) and inducing pro-tumor angiogenesis (132) (**Figure 2**). In the TME, MDSCs, by production of VEGF, FGF2, Bv8, and matrix metalloprotease (MMP) 9 (MMP9), can trigger and sustain tumor angiogenesis (44, 133) (**Figure 3**). Co-injection of murine tumors with $CD11b^{+}Gr1^{+}$ MDSCs increased intra-tumor vascular density, reduced necrosis, and augmented tumor growth (133, 134). $CD11b^{+}Gr1^{+}$ MDSCs cells directly contribute to tumor angiogenesis by producing MMP9 or acquiring endothelial cell properties in TME (133). MDSCs may directly take part in the formation of tumor vasculature by being incorporated into the vessel wall (133, 135) (**Figure 3**). Several studies have linked MDSC accumulation with an increase in intra-tumor VEGF concentration during disease progression (136). Approaches aiming at reducing levels of circulating MDSCs or in the tumor milieu were associated with decreased angiogenesis and delayed tumor growth (132, 137).

MDSCs can boost angiogenesis and stimulate tumor neovasculature by producing high levels of MMPs (**Figure 2**), including MMP2, MMP8, MMP9, MMP13, and MMP14 (130, 133, 138). MDSCs from MMP9-knockout mice have a significant reduction in their tumor promoting activity (133). Previous research has indicated that MDSCs with high levels of MMP9, trigger VEGF function by raising its bioavailability (139). In a mouse melanoma model, MDSCs contributed to A2B adenosine receptor-induced VEGF production, increased vessel density and angiogenesis (140, 141).

VEGF in turn stimulates MDSC recruitment, creating a positive feedforward loop. Promoting immunosuppression and angiogenesis (142, 143). MDSCs stimulated by VEGF had stronger immunosuppressive properties than non-stimulated MDSCs (143). VEGF-induced MDSCs stimulate the expansion of other immunosuppressive cells, including $FOXP3^{+}$ Tregs, through a TGFβ-dependent and/or independent pathway (143–145). The relationship between development of resistance to anti-angiogenic therapy with significant MDSC infiltration have been widely demonstrated in several studies (146–148). In agreement with these findings, MDSCs ablation has been reported to have synergistic effects with anti-VEGF/VEGFR treatment in refractory tumors (130, 143). It is now widely accepted that MDSCs interfere with the efficacy of VEGF-targeted therapy, either by secreting large quantities of VEGF that overcome VEGF inhibition, or by activating VEGF-independent pro-angiogenic signaling pathways (149).

The expression of VEGF, MDSCs can modify the TME in a pro-angiogenic manner through the production of several other angiogenic factors and also chemokines which can further enhance MDSCs accumulation within tumors, creating a vicious circle. CCL2, CXCL8, CXCL2, IL-1β, angiopoietin 1 and 2, and GM-CSF have been shown to contribute to MDSC-mediated

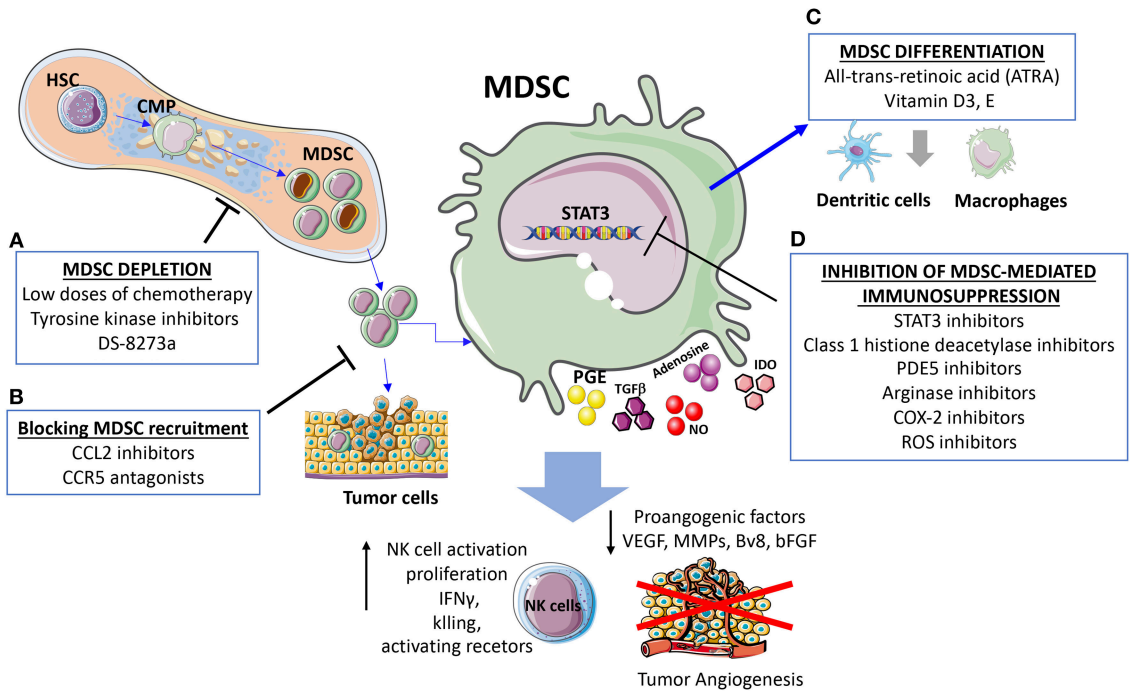

FIGURE 3 | Strategies for targeting MDSC and cross-talk with NK. The presented strategies act simultaneously on MDSC and MDSC-released factors dampening NK cell immunosuppression and induction of angiogenesis. **(A)** MDSC depletion can be induced using low doses of chemotherapy, tyrosine kinase inhibitors, the anti-DR5 monoclonal antibody DS-8273a. **(B)** Strategies blocking MDSC recruitments by CCL2 inhibitors and CCR5 antagonists. **(C)** Differentiation of MDSC into non-immunosuppressive cells induced by all-trans-retinoic acid (ATRA), Vitamin D3, and Vitamin E. **(D)** Inhibition of MDSC immunosuppression can be induced by STAT3 inhibitors, reduction of immunosuppressive agents such as Arginase and ROS, along with attenuation of the inflammatory state.

angiogenesis and require STAT3 for their expression (4, 150–152) (**Figure 2**). Anti-CCL2 treatment decrease PMN-MDSC and M-MDSC and reduce endothelial cell migration (150, 153, 154). MDSCs promote angiogenesis also via expression of a prokineticin 2, known as Bv8, which plays an important role in myeloid cell-mediated tumor angiogenesis (155). A refractory behavior to anti-VEGF therapy was associated with high number of $CD11b^{+}Gr1^{+}$ cells expressing Bv8 in peripheral blood and tumor (156). Thus, it has been suggested that combination of anti-Bv8 antibodies and anti-VEGF may better inhibit angiogenesis and control the tumor growth in anti-VEGF refractory tumors (156, 157). A close expression among molecules associated with angiogenesis: p-STAT3, VEGFA, CK2, and the MDSCs marker CD11b was found in head and neck squamous cell carcinoma (HNSCC) patients (158). Inhibition of JAK2/STAT3 in HNSCC transgenic mouse model reduced MDSC number and suppressed angiogenesis by decreasing VEGFA and hypoxia inducible factor (HIF-1α) both *in vitro* and *vivo* (158) (**Figure 2**).

Hypoxia, which is a feature of tumor bearing TME, has a crucial role in stimulating HIF-1α mediated signaling. HIF-1 and/or HIF-2 create a proangiogenic TME by inducing the expression of proangiogenic factors (VEGF, ANG-2, PlGF, bFGF, and semaphorin 4D). It was shown that in myeloid cells, HIF-1 activation promotes angiogenesis through VEGF and S100A8 (159) and lead to accumulation of MDSCs positive for the expression of CX3CR1, a CCL26 receptor, in hypoxic tumor regions (111, 160) (**Figure 2**).

ROS (radical oxygen species) also play an important role in the expansion of MDSCs and augmented levels of these molecules have been shown to stimulate the expression of VEGF receptors on MDSCs and their recruitment in the TME (142, 161).

STRATEGIES TO TARGET MDSCs AND INTERFERE WITH NK CROSSTALK

The main strategies to target MDSC and consequently their crosstalk with NK cells include: (i) regulation of myelopoiesis and MDSC depletion (tyrosine kinase inhibitors, cytotoxic agents), (ii) enhancement of MDSC differentiation (ATRA, Vitamin A, D3), (iii) inhibition of MDSC recruitment at the site of tumor (CCR5 antagonist, CCL2 inhibitor) (132, 162), (iv) inhibition of MDSC-mediated immunosuppression (STAT3 inhibitors, PDE5, histone deacetylase, NO inhibitors, Arginase inhibitors, ROS inhibitors, COX-2 inhibitors, phenformin, metformin, Polyinosinic-polycytidylic acid) (**Figure 3**). Here, we will briefly discuss strategies to target MDSC immunosuppression and the effects on angiogenesis and NK cell.

Recent work has provided evidence that relatively low doses of chemotherapy induce MDSC exhaustion (22, 163). Gemcitabine (164), Lurbinectedin (PM01183) (165) 5-azacytidine (166),

docetaxel (167), paclitaxel (168), 5-Fluorouracil (169), and doxorubicin (170) exert beneficial effects by reducing MDSC frequencies, increasing responsiveness to immune therapy and enhancing the antitumor activity of activated NK cells (171–174). Similarly, tyrosine kinase inhibitors such as Axitinib, sunitinib, and brutinib, directly target VEGF and/or c-KIT signaling, interfering with tumor-driven expansion MDSC factors such as M-CSF and STAT3 (175–181). In addition to angiogenesis inhibition, sunitinib treatment upregulates NKG2DLs and induces higher cytotoxic sensitivity of tumor cells to NK cells (182–184).

Several studies reported that vitamins D3, A, and E decrease levels of immature MDSC leading to improved anti-tumor activity in the context of immunotherapeutic interventions (185, 186). Vitamin D insufficient and deficient patients had lower NK-mediated cytotoxicity (187), whereas vitamin D receptor (VDR) agonist inhibited selectively ocular hyaloid vasculature angiogenesis in zebrafish models (188). Vitamin E enhance immune responses via reducing ROS levels and inhibition of PGE_2, COX2, activity mediated through decreasing NO production (189). MDSCs impair NK cell function via production of NO (92), thus, its inhibition offers a strategy for targeting MDSC-NK crosstalk. Promising results on reducing MDSC frequency or increasing their differentiation, were obtained in clinical trials using vitamin A metabolite, *all* -trans-retinoic acid (ATRA), tested alone (190–192) or in combination with IL-2 administration (191) or with a DC vaccine against p53 (193). In preclinical breast cancer models, ATRA improved antiangiogenic therapies by reverting the anti-VEGFR2-induced accumulation of intratumoral MDSCs, decreased hypoxia, and interfered with the disorganization of tumor microvessels (194). Similarly, it was shown that ATRA, suppresses the angiopoietin-Tie2 pathway, inhibits angiogenesis and progression of esophageal squamous xenograft tumors (195). ATRA increased the expression of MICA and MICB in tumor cells, promoting NK cell activation (175, 196), although other studies reported contrasting effects (197, 198).

Blockade of MDSC recruitment at the tumor site inhibits the establishment of an immunosuppressive pre-metastatic niche, via MDSC suppression of NK cells (64). Blocking CCR5/CCR5 ligand interaction by using fusion protein mCCR5–Ig-neutralizing CCR5 ligands, reduced migration, and immunosuppressive potential of MDSCs in the TME and significantly improved survival of tumor-bearing mice (199). In addition, blocking CCL2, which is produced by MDSCs, using specific antibodies can reduce angiogenesis by blocking endothelial cell migration (153).

STAT3 pharmacological inhibition (by peptidomimetics, small molecule inhibitors, platinum agents, curcumin, JAK inhibitors, AG490, Cucurbitacin B) simultaneously blocks angiogenesis and accumulation/suppressive function of MDSC, neutralizing the induction of a tolerogenic/tumor permissive TME, without MDSC depletion (158, 200–202).

JAK/STAT3 inhibitors suppress angiogenesis and reduce MDSCs in the TME through VEGFA and CK2 inhibition (158). Several studies demonstrated a link between STAT3 blockade, TGFβ inhibition and increased tumor surveillance by NK cells (80, 81). Peripheral and tumor-associated NK cells in STAT3-targeted tumor-bearing mice, exhibit higher expression of the NK activation markers NKG2D, CD69, Fas ligand (FasL), granzyme B, perforin, and IFNγ, resulting in reduced tumor growth and enhanced survival (80, 81). Given the STAT3 inhibitors side effects, a STAT3siRNA or decoy STAT3 oligonucleotide inhibitors, such as AZD9150 have been recently developed and combined with immune checkpoint inhibitors, in phase I/II clinical trials (203–205). In similar approach, STAT3 siRNA or decoy oligonucleotides, coupled to CpG oligonucleotides, have been employed to ensure a selective delivery of the drugs to TLR9-expressing myeloid cells (in particular, PMN-MDSC), displaying a decreased immunosuppressive activity (203). Therefore, STAT3 inhibitors provide a potential strategy to reduce immunosuppression activate NK cells and reduce angiogenesis (4).

Class I histone deacetylase inhibitor, entinostat, has been reported to inhibit the immunosuppressive function of MDSC by reducing ARG1, iNOS, and COX2 levels in both M- and PMN-MDSC subsets (206, 207). Vorinostat and entinostat significantly enhanced the expression of multiple NK ligands and death receptors, resulting in enhanced NK cell-mediated cytotoxicity (208).

Several clinical and preclinical mouse model studies, employing PDE-5 inhibitors, such as sildenafil and tadalafil, have demonstrated decreased MDSC accumulation and their immunosuppressive pattern functions by inhibiting iNOS, ARG1, IL4Ra, ROS levels and enabling NK cell anti-tumor cytotoxicity together with activation of anti-tumor response resulting in improved clinical outcome of advanced cancer patients (60, 209–215).

Arginase inhibitors are promising pharmacological agents to treat NK suppression (216) and blocking Arg1 activity in the TME could shift the balance of L-arginine metabolism, favoring T cell and NK cell proliferation (217). In murine studies, injection of the arginase inhibitor hydroxy-nor-arginine (nor-NOHA) or Nω-hydroxy-arginine (NOHA) or genetic disruption of *Arg1* in the myeloid compartment resulted in reduced tumor growth (218–220). In murine syngeneic tumor model, CB-1158, a potent and orally-bioavailable small-molecule inhibitor of arginase, shifted the tumor immune landscape blunting myeloid cell-mediated immune evasion, increasing tumor-infiltrating $CD8^+$ T cells and NK cells (182). In colorectal cancer patients undergoing tumor resection, supplementation of arginine prior to surgery resulted in an increase in $CD16^+$ and $CD56^+$ NK cells infiltrating the tumors (221).

Cyclooxygenase (COX)-2 inhibitors, celecoxib, or nimesulide have been successfully tested in preclinical models for preventing local and systemic expansion of all MDSC subtypes resulting in reduced tumor progression (222–225). On the hand, COX-2 inhibitors induce the expression of NKG2D ligands in cancer cells and increase their susceptibility to NK cell-mediated cell death (226, 227) together with blocking multiple angiogenic and lymphangiogenic such as VEGF-A, VEGF-C/D) (228).

ROS production is one the mechanisms employed by MDSC for immunosuppression (226, 229). In this context, phytochemicals, via their antioxidant property, can activate Nrf2

pathway, that is considered tumor-protective, in particular in the early stages of tumorigenesis. The synthetic triterpenoid C-28 methyl ester of 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid (CDDO-Me, also referred to as bardoxolone methyl, RTA402, TP-155, and NSC713200) is a potent Nrf2 activator and has been found to reduce MDSC production of ROS and tumor growth in mouse tumor models (230) and showed a promising anticancer effect in a phase I trial (231). In addition, Nrf2 upregulation, regulates early anti-cancer immune responses and induces the cytokine interleukin-17D (IL-17D), that is overexpressed in highly immunogenic tumor cells and play an important role in immune rejection mediated by NK cells (232, 233). Inducing IL-17D using Nrf2 agonists boost innate immunity and NK recruitment leading to tumor-regression (234, 235). An increasing number of recent reports suggest the abilities of the antidiabetic drugs, phenformin, and metformin to selectively reduce the number -MDSCs and the immunosuppressive functions of MDSC in the TME, through the activation of AMPK (236–240). Phenformin and metformin were able to inhibit immune suppressive activities MDSCs and potentiated the anti-tumor activity of PD-1 blockade immunotherapy (236, 240, 241).

In addition, metformin and phenformin have been widely investigated for their properties in inhibiting angiogenesis and blocking tumor progression (242–244). Several scientific evidences revealed that metformin exerts also strong immunomodulatory effects and contributes to the enhancement of cytotoxic T lymphocyte (245–247) *Polyinosinic-polycytidylic acid [Poly (I: C)* an agonist for pattern-recognition receptors (PRRs), toll-like receptor 3 (TLR3) has been reported to decrease MDSC frequencies in BM, blood, and tumor and abrogate their immunosuppressive, concomitant with an NK cell activation (248–251).

CONCLUSIONS

MDSC are major players in the immunosuppressive scenario in cancer, thanks to their phenotype heterogeneity and critical interaction with several innate immune cells, thus representing a crucial target in oncology. Here we reviewed the interactions of MDSCs with NK cells. The contribution of key cytokines, chemokines and mediators active in this process have been discussed.

We also described the contribution of MDSC on angiogenesis directly or indirectly through interactions with NK and immunosuppressive activities. A parallel of the cancer associated to the decidual counterpart of these cells is discussed, as to propose an onco-fetal origin of the polarization.

In addition to the well-characterized role in immunosuppression, MDSC possess potent pro-angiogenic capabilities, and actively participate in the resistance to VEGF-targeted therapy. Considering the crucial role of MDSC in inducing and regulating a permissive immune TME, in directly contributing to angiogenesis and tumor invasion, several strategies to therapeutically target these cells are currently being tested in clinic. Several pre-clinical studies show that targeting MDSC through multiple approaches helps to increases NK cells tumor activity augment the efficacy of anti-angiogenic therapy.

A better understanding of the link between MDSC-NK immunosuppressive network in TME and their influence on angiogenesis can be translated to new therapeutic targets.

AUTHOR CONTRIBUTIONS

DN and AA: design, review and revision of the manuscript, and revision of the figures; LM: writing, review, and revision of the manuscript; DB: writing, review, and preparing figures; AB: design, writing, review, and revision of the manuscript and drafting of the figures.

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REFERENCES

1. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci.* (2012) 125 (Pt 23):5591–6. doi: 10.1242/jcs.116392
2. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med.* (2018) 24:541–50. doi: 10.1038/s41591-018-0014-x
3. Noonan DM, De Lerma Barbaro A, Vannini N, Mortara L, Albini A. Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. *Cancer Metastasis Rev.* (2008) 27:31–40. doi: 10.1007/s10555-007-9108-5
4. Albini A, Bruno A, Noonan DM, Mortara L. Contribution to tumor angiogenesis from innate immune cells within the tumor microenvironment: implications for immunotherapy. *Front Immunol.* (2018) 9:527. doi: 10.3389/fimmu.2018.00527
5. Granot Z, Fridlender ZG. Plasticity beyond cancer cells and the "immunosuppressive switch". *Cancer Res.* (2015) 75:4441–5. doi: 10.1158/0008-5472.CAN-15-1502
6. Holzel M, Bovier A, Tuting T. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? *Nat Rev Cancer.* (2013) 13:365–76. doi: 10.1038/nrc3498
7. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Semin Cancer Biol.* (2012) 22:275–81. doi: 10.1016/j.semcancer.2012.01.011
8. Sinha P, Clements VK, Bunt SK, Albelda SM, Ostrand-Rosenberg S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J Immunol.* (2007) 179:977–83. doi: 10.4049/jimmunol.179.2.977
9. Ben-Meir K, Twaik N, Baniyash M. Plasticity and biological diversity of myeloid derived suppressor cells. *Curr Opin Immunol.* (2018) 51:154–61. doi: 10.1016/j.coi.2018.03.015
10. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer.* (2019) 120:16–25. doi: 10.1038/s41416-018-0333-1
11. Umansky V, Blattner C, Fleming V, Hu X, Gebhardt C, Altevogt P, et al. Myeloid-derived suppressor cells and tumor escape from

immune surveillance. *Semin Immunopathol.* (2017) 39:295–305. doi: 10.1007/s00281-016-0597-6
12. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol.* (2008) 9:503–10. doi: 10.1038/ni1582
13. Baginska J, Viry E, Paggetti J, Medves S, Berchem G, Moussay E, et al. The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity. *Front Immunol.* (2013) 4:490. doi: 10.3389/fimmu.2013.00490
14. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol.* (2016) 17:1025–36. doi: 10.1038/ni.3518
15. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. *Oncogene.* (2008) 27:5932–43. doi: 10.1038/onc.2008.267
16. Bosi A, Zanellato S, Bassani B, Albini A, Musco A, Cattoni M, et al. Natural killer cells from malignant pleural effusion are endowed with a decidual-like proangiogenic polarization. *J Immunol Res.* (2018) 2018:2438598. doi: 10.1155/2018/2438598
17. Bruno A, Ferlazzo G, Albini A, Noonan DM. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. *J Natl Cancer Inst.* (2014) 106:dju200. doi: 10.1093/jnci/dju200
18. Gotthardt D, Putz EM, Grundschober E, Prchal-Murphy M, Straka E, Kudweis P, et al. STAT5 is a key regulator in NK cells and acts as a molecular switch from tumor surveillance to tumor promotion. *Cancer Discov.* (2016) 6:414–29. doi: 10.1158/2159-8290.CD-15-0732
19. Levi I, Amsalem H, Nissan A, Darash-Yahana M, Peretz T, Mandelboim O, et al. Characterization of tumor infiltrating natural killer cell subset. *Oncotarget.* (2015) 6:13835–43. doi: 10.18632/oncotarget.3453
20. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
21. Budhwar S, Verma P, Verma R, Rai S, Singh K. The Yin and Yang of myeloid derived suppressor cells. *Front Immunol.* (2018) 9:2776. doi: 10.3389/fimmu.2018.02776
22. Draghiciu O, Lubbers J, Nijman HW, Daemen T. Myeloid derived suppressor cells-An overview of combat strategies to increase immunotherapy efficacy. *Oncoimmunology.* (2015) 4:e954829. doi: 10.4161/21624011.2014.954829
23. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res.* (2017) 5:3–8. doi: 10.1158/2326-6066.CIR-16-0297
24. Sica A, Massarotti M. Myeloid suppressor cells in cancer and autoimmunity. *J Autoimmun.* (2017) 85:117–25. doi: 10.1016/j.jaut.2017.07.010
25. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells–a proposal for uniform nomenclature. *Nat Rev Immunol.* (2013) 13:145–9. doi: 10.1038/nri3365
26. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells: 10 years on. *Cell.* (2018) 174:1054–66. doi: 10.1016/j.cell.2018.07.017
27. Chalifour A, Jeannin P, Gauchat JF, Blaecke A, Malissard M, N'Guyen T, et al. Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers alpha-defensin production. *Blood.* (2004) 104:1778–83. doi: 10.1182/blood-2003-08-2820
28. Bouzani M, Ok M, McCormick A, Ebel F, Kurzai O, Morton CO, et al. Human NK cells display important antifungal activity against Aspergillus fumigatus, which is directly mediated by IFN-gamma release. *J Immunol.* (2011) 187:1369–76. doi: 10.4049/jimmunol.1003593
29. Li SS, Kyei SK, Timm-McCann M, Ogbomo H, Jones GJ, Shi M, et al. The NK receptor NKp30 mediates direct fungal recognition and killing and is diminished in NK cells from HIV-infected patients. *Cell Host Microbe.* (2013) 14:387–97. doi: 10.1016/j.chom.2013.09.007
30. Murphy WJ, Bennett M, Kumar V, Longo DL. Donor-type activated natural killer cells promote marrow engraftment and B cell development during allogeneic bone marrow transplantation. *J Immunol.* (1992) 148:2953–60.
31. Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. *Nature.* (2011) 481:394–8. doi: 10.1038/nature10624
32. Kumar P, Thakar MS, Ouyang W, Malarkannan S. IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection. *Mucosal Immunol.* (2013) 6:69–82. doi: 10.1038/mi.2012.49
33. Carrega P, Bonaccorsi I, Di Carlo E, Morandi B, Paul P, Rizzello V, et al. CD56(bright)perforin(low) noncytotoxic human NK cells are abundant in both healthy and neoplastic solid tissues and recirculate to secondary lymphoid organs via afferent lymph. *J Immunol.* (2014) 192:3805–15. doi: 10.4049/jimmunol.1301889
34. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol.* (2018) 18:671–88. doi: 10.1038/s41577-018-0061-z
35. Lopez-Soto A, Gonzalez S, Smyth MJ, Galluzzi L. Control of Metastasis by NK Cells. *Cancer Cell.* (2017) 32:135–54. doi: 10.1016/j.ccell.2017.06.009
36. Parisi L, Bassani B, Tremolati M, Gini E, Farronato G, Bruno A. Natural killer cells in the orchestration of chronic inflammatory diseases. *J Immunol Res.* (2017) 2017:4218254. doi: 10.1155/2017/4218254
37. Wu J, Lanier LL. Natural killer cells and cancer. *Adv Cancer Res.* (2003) 90:127–56. doi: 10.1016/S0065-230X(03)90004-2
38. Zimmer J. Immunotherapy: natural killers take on cancer. *Nature.* (2014) 505:483. doi: 10.1038/505483e
39. Bruno A, Focaccetti C, Pagani A, Imperatori AS, Spagnoletti M, Rotolo N, et al. The proangiogenic phenotype of natural killer cells in patients with non-small cell lung cancer. *Neoplasia.* (2013) 15:133–42. doi: 10.1593/neo.121758
40. Bruno A, Bassani B, D'Urso DG, Pitaku I, Cassinotti E, Pelosi G, et al. Angiogenin and the MMP9-TIMP2 axis are up-regulated in proangiogenic, decidual NK-like cells from patients with colorectal cancer. *FASEB J.* (2018) 32:5365–77. doi: 10.1096/fj.201701103R
41. Mandruzzato S, Brandau S, Britten CM, Bronte V, Damuzzo V, Gouttefangeas C, et al. Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry: results from an interim study. *Cancer Immunol Immunother.* (2016) 65:161–9. doi: 10.1007/s00262-015-1782-5
42. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol.* (2016) 1:aaf8943. doi: 10.1126/sciimmunol.aaf8943
43. Condamine T, Mastio J, Gabrilovich DI. Transcriptional regulation of myeloid-derived suppressor cells. *J Leukoc Biol.* (2015) 98:913–22. doi: 10.1189/jlb.4RI0515-204R
44. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol.* (2016) 37:208–20. doi: 10.1016/j.it.2016.01.004
45. Messmer MN, Netherby CS, Banik D, Abrams SI. Tumor-induced myeloid dysfunction and its implications for cancer immunotherapy. *Cancer Immunol Immunother.* (2015) 64:1–13. doi: 10.1007/s00262-014-1639-3
46. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann N Y Acad Sci.* (2014) 1319:47–65. doi: 10.1111/nyas.12469
47. Zhang S, Ma X, Zhu C, Liu L, Wang G, Yuan X. The role of myeloid-derived suppressor cells in patients with solid tumors: a meta-analysis. *PLoS ONE.* (2016) 11:e0164514. doi: 10.1371/journal.pone.0164514
48. Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM. Mechanism of immune dysfunction in cancer mediated by immature Gr-1^{+} myeloid cells. *J Immunol.* (2001) 166:5398–406. doi: 10.4049/jimmunol.166.9.5398
49. Melani C, Chiodoni C, Forni G, Colombo MP. Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. *Blood.* (2003) 102:2138–45. doi: 10.1182/blood-2003-01-0190
50. Monu NR, Frey AB. Myeloid-derived suppressor cells and anti-tumor T cells: a complex relationship. *Immunol Invest.* (2012) 41:595–613. doi: 10.3109/08820139.2012.673191
51. Hoechst B, Gamrekelashvili J, Manns MP, Greten TF, Korangy F. Plasticity of human Th17 cells and iTregs is orchestrated by different subsets of myeloid cells. *Blood.* (2011) 117:6532–41. doi: 10.1182/blood-2010-11-317321
52. Elkabets M, Ribeiro VS, Dinarello CA, Ostrand-Rosenberg S, Di Santo JP, Apte RN, et al. IL-1beta regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *Eur J Immunol.* (2010) 40:3347–57. doi: 10.1002/eji.201041037

53. Liu C, Yu S, Kappes J, Wang J, Grizzle WE, Zinn KR, et al. Expansion of spleen myeloid suppressor cells represses NK cell cytotoxicity in tumor-bearing host. *Blood.* (2007) 109:4336–42. doi: 10.1182/blood-2006-09-046201
54. Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, et al. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood.* (2008) 111:4233–44. doi: 10.1182/blood-2007-07-099226
55. Allan DS, Rybalov B, Awong G, Zuniga-Pflucker JC, Kopcow HD, Carlyle JR, et al. TGF-beta affects development and differentiation of human natural killer cell subsets. *Eur J Immunol.* (2010) 40:2289–95. doi: 10.1002/eji.200939910
56. Cerdeira AS, Rajakumar A, Royle CM, Lo A, Husain Z, Thadhani RI, et al. Conversion of peripheral blood NK cells to a decidual NK-like phenotype by a cocktail of defined factors. *J Immunol.* (2013) 190:3939–48. doi: 10.4049/jimmunol.1202582
57. Keskin DB, Allan DS, Rybalov B, Andzelm MM, Stern JN, Kopcow HD, et al. TGFbeta promotes conversion of $CD16^+$ peripheral blood NK cells into $CD16^-$ NK cells with similarities to decidual NK cells. *Proc Natl Acad Sci USA.* (2007) 104:3378–83. doi: 10.1073/pnas.0611098104
58. Kopcow HD, Allan DS, Chen X, Rybalov B, Andzelm MM, Ge B, et al. Human decidual NK cells form immature activating synapses and are not cytotoxic. *Proc Natl Acad Sci USA.* (2005) 102:15563–8. doi: 10.1073/pnas.0507835102
59. Li H, Han Y, Guo Q, Zhang M, Cao X. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. *J Immunol.* (2009) 182:240–9. doi: 10.4049/jimmunol.182.1.240
60. Tai LH, Alkayyal AA, Leslie AL, Sahi S, Bennett S, Tanese de Souza C, et al. Phosphodiesterase-5 inhibition reduces postoperative metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. *Oncoimmunology.* (2018) 7:e1431082. doi: 10.1080/2162402X.2018.1431082
61. Vaknin I, Blinder L, Wang L, Gazit R, Shapira E, Genina O, et al. A common pathway mediated through Toll-like receptors leads to T- and natural killer-cell immunosuppression. *Blood.* (2008) 111:1437–47. doi: 10.1182/blood-2007-07-100404
62. Hoechst B, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, Wedemeyer H, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology.* (2009) 50:799–807. doi: 10.1002/hep.23054
63. Nausch N, Galani IE, Schlecker E, Cerwenka A. Mononuclear myeloid-derived "suppressor" cells express RAE-1 and activate natural killer cells. *Blood.* (2008) 112:4080–9. doi: 10.1182/blood-2008-03-143776
64. Sceneay J, Chow MT, Chen A, Halse HM, Wong CS, Andrews DM, et al. Primary tumor hypoxia recruits $CD11b^+$/Ly6Cmed/$Ly6G^+$ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche. *Cancer Res.* (2012) 72:3906–11. doi: 10.1158/0008-5472.CAN-11-3873
65. Binsfeld M, Muller J, Lamour V, De Veirman K, De Raeve H, Bellahcene A, et al. Granulocytic myeloid-derived suppressor cells promote angiogenesis in the context of multiple myeloma. *Oncotarget.* (2016) 7:37931–43. doi: 10.18632/oncotarget.9270
66. Condamine T, Ramachandran I, Youn JI, Gabrilovich DI. Regulation of tumor metastasis by myeloid-derived suppressor cells. *Annu Rev Med.* (2015) 66:97–110. doi: 10.1146/annurev-med-051013-052304
67. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest.* (2015) 125:3356–64. doi: 10.1172/JCI80005
68. Fortin C, Huang X, Yang Y. NK cell response to vaccinia virus is regulated by myeloid-derived suppressor cells. *J Immunol.* (2012) 189:1843–9. doi: 10.4049/jimmunol.1200584
69. Tai LH, de Souza CT, Belanger S, Ly L, Alkayyal AA, Zhang J, et al. Preventing postoperative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells. *Cancer Res.* (2013) 73:97–107. doi: 10.1158/0008-5472.CAN-12-1993
70. Angka L, Khan ST, Kilgour MK, Xu R, Kennedy MA, Auer RC. Dysfunctional natural killer cells in the aftermath of cancer surgery. *Int J Mol Sci.* (2017) 18:18081787. doi: 10.3390/ijms18081787
71. Ihle JN. The Stat family in cytokine signaling. *Curr Opin Cell Biol.* (2001) 13:211–7. doi: 10.1016/S0955-0674(00)00199-X
72. Murray PJ. The JAK-STAT signaling pathway: input and output integration. *J Immunol.* (2007) 178:2623–9. doi: 10.4049/jimmunol.178.5.2623
73. O'Shea JJ, Gadina M, Kanno Y. Cytokine signaling: birth of a pathway. *J Immunol.* (2011) 187:5475–8. doi: 10.4049/jimmunol.1102913
74. O'Shea JJ, Holland SM, Staudt LM. JAKs and STATs in immunity, immunodeficiency, and cancer. *N Engl J Med.* (2013) 368:161–70. doi: 10.1056/NEJMra1202117
75. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol.* (2018) 15:234–48. doi: 10.1038/nrclinonc.2018.8
76. Thomas SJ, Snowden JA, Zeidler MP, Danson SJ. The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours. *Br J Cancer.* (2015) 113:365–71. doi: 10.1038/bjc.2015.233
77. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer.* (2009) 9:798–809. doi: 10.1038/nrc2734
78. Sucher R, Kurz K, Weiss G, Margreiter R, Fuchs D, Brandacher G. IDO-mediated tryptophan degradation in the pathogenesis of malignant tumor disease. *Int J Tryptophan Res.* (2010) 3:113–20. doi: 10.4137/IJTR.S4157
79. Yu J, Wang Y, Yan F, Zhang P, Li H, Zhao H, et al. Noncanonical NF-kappaB activation mediates STAT3-stimulated IDO upregulation in myeloid-derived suppressor cells in breast cancer. *J Immunol.* (2014) 193:2574–86. doi: 10.4049/jimmunol.1400833
80. Sun X, Sui Q, Zhang C, Tian Z, Zhang J. Targeting blockage of STAT3 in hepatocellular carcinoma cells augments NK cell functions via reverse hepatocellular carcinoma-induced immune suppression. *Mol Cancer Ther.* (2013) 12:2885–96. doi: 10.1158/1535-7163.MCT-12-1087
81. Sui Q, Zhang J, Sun X, Zhang C, Han Q, Tian Z. NK cells are the crucial antitumor mediators when STAT3-mediated immunosuppression is blocked in hepatocellular carcinoma. *J Immunol.* (2014) 193:2016–23. doi: 10.4049/jimmunol.1302389
82. Gotthardt D, Sexl V. STATs in NK-cells: the good, the bad, and the ugly. *Front Immunol.* (2016) 7:694. doi: 10.3389/fimmu.2016.00694
83. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* (2012) 12:253–68. doi: 10.1038/nri3175
84. Bierie B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer.* (2006) 6:506–20. doi: 10.1038/nrc1926
85. Lebrun JJ. The dual role of TGFbeta in human cancer: from tumor suppression to cancer metastasis. *ISRN Mol Biol.* (2012) 2012:381428. doi: 10.5402/2012/381428
86. Massague J. TGFbeta in cancer. *Cell.* (2008) 134:215–30. doi: 10.1016/j.cell.2008.07.001
87. Molgora M, Bonavita E, Ponzetta A, Riva F, Barbagallo M, Jaillon S, et al. IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. *Nature.* (2017) 551:110–4. doi: 10.1038/nature24293
88. Fournie JJ, Poupot M. The pro-tumorigenic IL-33 involved in antitumor immunity: a Yin and Yang cytokine. *Front Immunol.* (2018) 9:2506. doi: 10.3389/fimmu.2018.02506
89. Shen JX, Liu J, Zhang GJ. Interleukin-33 in malignancies: friends or foes? *Front Immunol.* (2018) 9:3051. doi: 10.3389/fimmu.2018.03051
90. Choudhari SK, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: a review. *World J Surg Oncol.* (2013) 11:118. doi: 10.1186/1477-7819-11-118
91. Ying L, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res.* (2007) 67:1407–10. doi: 10.1158/0008-5472.CAN-06-2149
92. Stiff A, Trikha P, Mundy-Bosse B, McMichael E, Mace TA, Benner B, et al. Nitric oxide production by myeloid-derived suppressor cells plays a role in impairing Fc receptor-mediated natural killer cell function. *Clin Cancer Res.* (2018) 24:1891–904. doi: 10.1158/1078-0432.CCR-17-0691
93. Cifone MG, D'Alo S, Parroni R, Millimaggi D, Biordi L, Martinotti S, et al. Interleukin-2-activated rat natural killer cells express inducible nitric oxide synthase that contributes to cytotoxic function and interferon-gamma production. *Blood.* (1999) 93:3876–84.

94. Furuke K, Burd PR, Horvath-Arcidiacono JA, Hori K, Mostowski H, Bloom ET. Human NK cells express endothelial nitric oxide synthase, and nitric oxide protects them from activation-induced cell death by regulating expression of TNF-alpha. *J Immunol.* (1999) 163:1473–80.
95. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. *Trends Immunol.* (2002) 23:144–50. doi: 10.1016/S1471-4906(01)02154-8
96. Maas R, Heller M. [Computed tomography of the skeletal system]. *Rontgenpraxis.* (1990) 43:293–300.
97. Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer.* (2010) 10:181–93. doi: 10.1038/nrc2809
98. Mao Y, Sarhan D, Steven A, Seliger B, Kiessling R, Lundqvist A. Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. *Clin Cancer Res.* (2014) 20:4096–106. doi: 10.1158/1078-0432.CCR-14-0635
99. Newson J, Motwani MP, Kendall AC, Nicolaou A, Muccioli GG, Alhouayek M, et al. Inflammatory resolution triggers a prolonged phase of immune suppression through COX-1/mPGES-1-derived prostaglandin E2. *Cell Rep.* (2017) 20:3162–75. doi: 10.1016/j.celrep.2017.08.098
100. Lob S, Konigsrainer A, Rammensee HG, Opelz G, Terness P. Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees? *Nat Rev Cancer.* (2009) 9:445–52. doi: 10.1038/nrc2639
101. Munn DH, Mellor AL. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. *Trends Immunol.* (2016) 37:193–207. doi: 10.1016/j.it.2016.01.002
102. Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, et al. Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. *Proc Natl Acad Sci USA.* (2013) 110:E231–40. doi: 10.1073/pnas.1206322110
103. Godin-Ethier J, Pelletier S, Hanafi LA, Gannon PO, Forget MA, Routy JP, et al. Human activated T lymphocytes modulate IDO expression in tumors through Th1/Th2 balance. *J Immunol.* (2009) 183:7752–60. doi: 10.4049/jimmunol.0901004
104. Gostner JM, Becker K, Uberall F, Fuchs D. The potential of targeting indoleamine 2,3-dioxygenase for cancer treatment. *Expert Opin Ther Targets.* (2015) 19:605–15. doi: 10.1517/14728222.2014.995092
105. Hornyak L, Dobos N, Koncz G, Karanyi Z, Pall D, Szabo Z, et al. The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. *Front Immunol.* (2018) 9:151. doi: 10.3389/fimmu.2018.00151
106. Yu J, Du W, Yan F, Wang Y, Li H, Cao S, et al. Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *J Immunol.* (2013) 190:3783–97. doi: 10.4049/jimmunol.1201449
107. Zhang J, Han X, Hu X, Jin F, Gao Z, Yin L, et al. IDO1 impairs NK cell cytotoxicity by decreasing NKG2D/NKG2DLs via promoting miR-18a. *Mol Immunol.* (2018) 103:144–55. doi: 10.1016/j.molimm.2018.09.011
108. Della Chiesa M, Carlomagno S, Frumento G, Balsamo M, Cantoni C, Conte R, et al. The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. *Blood.* (2006) 108:4118–25. doi: 10.1182/blood-2006-03-006700
109. Allard B, Beavis PA, Darcy PK, Stagg J. Immunosuppressive activities of adenosine in cancer. *Curr Opin Pharmacol.* (2016) 29:7–16. doi: 10.1016/j.coph.2016.04.001
110. Antonioli L, Blandizzi C, Pacher P, Hasko G. Immunity, inflammation and cancer: a leading role for adenosine. *Nat Rev Cancer.* (2013) 13:842–57. doi: 10.1038/nrc3613
111. Chiu DK, Tse AP, Xu IM, Di Cui J, Lai RK, Li LL, et al. Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma. *Nat Commun.* (2017) 8:517. doi: 10.1038/s41467-017-00530-7
112. Ryzhov SV, Pickup MW, Chytil A, Gorska AE, Zhang Q, Owens P, et al. Role of TGF-beta signaling in generation of $CD39^{+}CD73^{+}$ myeloid cells in tumors. *J Immunol.* (2014) 193:3155–64. doi: 10.4049/jimmunol.1400578
113. Raskovalova T, Lokshin A, Huang X, Jackson EK, Gorelik E. Adenosine-mediated inhibition of cytotoxic activity and cytokine production by IL-2/NKp46-activated NK cells: involvement of protein kinase A isozyme I (PKA I). *Immunol Res.* (2006) 36:91–9. doi: 10.1385/IR:36:1:91
114. Morandi F, Horenstein AL, Chillemi A, Quarona V, Chiesa S, Imperatori A, et al. CD56brightCD16- NK cells produce adenosine through a CD38-mediated pathway and act as regulatory cells inhibiting autologous $CD4^{+}$ T cell proliferation. *J Immunol.* (2015) 195:965–72. doi: 10.4049/jimmunol.1500591
115. Cekic C, Day YJ, Sag D, Linden J. Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. *Cancer Res.* (2014) 74:7250–9. doi: 10.1158/0008-5472.CAN-13-3583
116. Blois SM, Klapp BF, Barrientos G. Decidualization and angiogenesis in early pregnancy: unravelling the functions of DC and NK cells. *J Reprod Immunol.* (2011) 88:86–92. doi: 10.1016/j.jri.2010.11.002
117. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med.* (2006) 12:1065–74. doi: 10.1038/nm1452
118. Wallace AE, Fraser R, Gurung S, Goulwara SS, Whitley GS, Johnstone AP, et al. Increased angiogenic factor secretion by decidual natural killer cells from pregnancies with high uterine artery resistance alters trophoblast function. *Hum Reprod.* (2014) 29:652–60. doi: 10.1093/humrep/deu017
119. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, et al. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. *J Leukoc Biol.* (2006) 80:572–80. doi: 10.1189/jlb.0406250
120. Naruse K, Lash GE, Innes BA, Otun HA, Searle RF, Robson SC, et al. Localization of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitors for MMPs (TIMPs) in uterine natural killer cells in early human pregnancy. *Hum Reprod.* (2009) 24:553–61. doi: 10.1093/humrep/den408
121. Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R, et al. Human decidua and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases. *Mol Hum Reprod.* (2011) 17:637–52. doi: 10.1093/molehr/gar033
122. Clark MM, Chazara O, Sobel EM, Gjessing HK, Magnus P, Moffett A, et al. Human birth weight and reproductive immunology: testing for interactions between maternal and offspring KIR and HLA-C genes. *Hum Hered.* (2016) 81:181–93. doi: 10.1159/000456033
123. Colucci F. The role of KIR and HLA interactions in pregnancy complications. *Immunogenetics.* (2017) 69:557–65. doi: 10.1007/s00251-017-1003-9
124. Cartwright JE, James-Allan L, Buckley RJ, Wallace AE. The role of decidual NK cells in pregnancies with impaired vascular remodelling. *J Reprod Immunol.* (2017) 119:81–4. doi: 10.1016/j.jri.2016.09.002
125. Kostlin N, Kugel H, Spring B, Leiber A, Marme A, Henes M, et al. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur J Immunol.* (2014) 44:2582–91. doi: 10.1002/eji.201344200
126. Nair RR, Sinha P, Khanna A, Singh K. Reduced myeloid-derived suppressor cells in the blood and endometrium is associated with early miscarriage. *Am J Reprod Immunol.* (2015) 73:479–86. doi: 10.1111/aji.12351
127. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol.* (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
128. Papatriantafyllou M. Tumour immunology: MDSCs come at a cost. *Nat Rev Immunol.* (2011) 11:440–1. doi: 10.1038/nri3017
129. Wang Y, Liu Y, Shu C, Wan J, Shan Y, Zhi X, et al. Inhibition of pregnancy-associated granulocytic myeloid-derived suppressor cell expansion and arginase-1 production in preeclampsia. *J Reprod Immunol.* (2018) 127:48–54. doi: 10.1016/j.jri.2018.05.002
130. Safarzadeh E, Orangi M, Mohammadi H, Babaie F, Baradaran B. Myeloid-derived suppressor cells: important contributors to tumor progression and metastasis. *J Cell Physiol.* (2018) 233:3024–36. doi: 10.1002/jcp.26075
131. Baniyash M. Myeloid-derived suppressor cells as intruders and targets: clinical implications in cancer therapy. *Cancer Immunol Immunother.* (2016) 65:857–67. doi: 10.1007/s00262-016-1849-y
132. Fleming V, Hu X, Weber R, Nagibin V, Groth C, Altevogt P, et al. Targeting myeloid-derived suppressor cells to bypass tumor-induced immunosuppression. *Front Immunol.* (2018) 9:398. doi: 10.3389/fimmu.2018.00398

133. Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, et al. Expansion of myeloid immune suppressor Gr^+CD11b^+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell.* (2004) 6:409–21. doi: 10.1016/j.ccr.2004.08.031
134. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H. Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J Clin Invest.* (2008) 118:3367–77. doi: 10.1172/JCI35213
135. Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer.* (2008) 8:618–31. doi: 10.1038/nrc2444
136. Karakhanova S, Link J, Heinrich M, Shevchenko I, Yang Y, Hassenpflug M, et al. Characterization of myeloid leukocytes and soluble mediators in pancreatic cancer: importance of myeloid-derived suppressor cells. *Oncoimmunology.* (2015) 4:e998519. doi: 10.1080/2162402X.2014.998519
137. Guedez L, Jensen-Taubman S, Bourboulia D, Kwityn CJ, Wei B, Caterina J, et al. TIMP-2 targets tumor-associated myeloid suppressor cells with effects in cancer immune dysfunction and angiogenesis. *J Immunother.* (2012) 35:502–12. doi: 10.1097/CJI.0b013e3182619c8e
138. Yang L, Huang J, Ren X, Gorska AE, Chytil A, Aakre M, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits $Gr\text{-}1^+CD11b^+$ myeloid cells that promote metastasis. *Cancer Cell.* (2008) 13:23–35. doi: 10.1016/j.ccr.2007.12.004
139. Jacob A, Prekeris R. The regulation of MMP targeting to invadopodia during cancer metastasis. *Front Cell Dev Biol.* (2015) 3:4. doi: 10.3389/fcell.2015.00004
140. Sorrentino C, Miele L, Porta A, Pinto A, Morello S. Myeloid-derived suppressor cells contribute to A2B adenosine receptor-induced VEGF production and angiogenesis in a mouse melanoma model. *Oncotarget.* (2015) 6:27478–89. doi: 10.18632/oncotarget.4393
141. Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Blockade of A2b adenosine receptor reduces tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse model of melanoma. *Neoplasia.* (2013) 15:1400–9. doi: 10.1593/neo.131748
142. Jayaraman P, Parikh F, Lopez-Rivera E, Hailemichael Y, Clark A, Ma G, et al. Tumor-expressed inducible nitric oxide synthase controls induction of functional myeloid-derived suppressor cells through modulation of vascular endothelial growth factor release. *J Immunol.* (2012) 188:5365–76. doi: 10.4049/jimmunol.1103553
143. Horikawa N, Abiko K, Matsumura N, Hamanishi J, Baba T, Yamaguchi K, et al. Expression of vascular endothelial growth factor in ovarian cancer inhibits tumor immunity through the accumulation of myeloid-derived suppressor cells. *Clin Cancer Res.* (2017) 23:587–99. doi: 10.1158/1078-0432.CCR-16-0387
144. Voron T, Marcheteau E, Pernot S, Colussi O, Tartour E, Taieb J, et al. Control of the immune response by pro-angiogenic factors. *Front Oncol.* (2014) 4:70. doi: 10.3389/fonc.2014.00070
145. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. *Front Immunol.* (2018) 9:978. doi: 10.3389/fimmu.2018.00978
146. Piao Y, Liang J, Holmes L, Zurita AJ, Henry V, Heymach JV, et al. Glioblastoma resistance to anti-VEGF therapy is associated with myeloid cell infiltration, stem cell accumulation, and a mesenchymal phenotype. *Neuro Oncol.* (2012) 14:1379–92. doi: 10.1093/neuonc/nos158
147. Hao Z, Sadek I. Sunitinib: the antiangiogenic effects and beyond. *Onco Targets Ther.* (2016) 9:5495–505. doi: 10.2147/OTT.S112242
148. Finke J, Ko J, Rini B, Rayman P, Ireland J, Cohen P. MDSC as a mechanism of tumor escape from sunitinib mediated anti-angiogenic therapy. *Int Immunopharmacol.* (2011) 11:856–61. doi: 10.1016/j.intimp.2011.01.030
149. Ferrara N. Role of myeloid cells in vascular endothelial growth factor-independent tumor angiogenesis. *Curr Opin Hematol.* (2010) 17:219–24. doi: 10.1097/MOH.0b013e3283386660
150. Chun E, Lavoie S, Michaud M, Gallini CA, Kim J, Soucy G, et al. CCL2 promotes colorectal carcinogenesis by enhancing polymorphonuclear myeloid-derived suppressor cell population and function. *Cell Rep.* (2015) 12:244–57. doi: 10.1016/j.celrep.2015.06.024
151. Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P. PGE(2)-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. *Cancer Res.* (2011) 71:7463–70. doi: 10.1158/0008-5472.CAN-11-2449
152. Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). *Clin Cancer Res.* (2016) 22:3924–36. doi: 10.1158/1078-0432.CCR-15-2463
153. Boelte KC, Gordy LE, Joyce S, Thompson MA, Yang L, Lin PC. Rgs2 mediates pro-angiogenic function of myeloid derived suppressor cells in the tumor microenvironment via upregulation of MCP-1. *PLoS ONE.* (2011) 6:e18534. doi: 10.1371/journal.pone.0018534
154. Wang Y, Zhang X, Yang L, Xue J, Hu G. Blockade of CCL2 enhances immunotherapeutic effect of anti-PD1 in lung cancer. *J Bone Oncol.* (2018) 11:27–32. doi: 10.1016/j.jbo.2018.01.002
155. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature.* (2007) 450:825–31. doi: 10.1038/nature06348
156. Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M, et al. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci USA.* (2009) 106:6742–7. doi: 10.1073/pnas.0902280106
157. Xin H, Lu R, Lee H, Zhang W, Zhang C, Deng J, et al. G-protein-coupled receptor agonist BV8/prokineticin-2 and STAT3 protein form a feed-forward loop in both normal and malignant myeloid cells. *J Biol Chem.* (2013) 288:13842–9. doi: 10.1074/jbc.M113.450049
158. Liu JF, Deng WW, Chen L, Li YC, Wu L, Ma SR, et al. Inhibition of JAK2/STAT3 reduces tumor-induced angiogenesis and myeloid-derived suppressor cells in head and neck cancer. *Mol Carcinog.* (2018) 57:429–39. doi: 10.1002/mc.22767
159. Ahn GO, Seita J, Hong BJ, Kim YE, Bok S, Lee CJ, et al. Transcriptional activation of hypoxia-inducible factor-1 (HIF-1) in myeloid cells promotes angiogenesis through VEGF and S100A8. *Proc Natl Acad Sci USA.* (2014) 111:2698–703. doi: 10.1073/pnas.1320243111
160. Chiu DK, Xu IM, Lai RK, Tse AP, Wei LL, Koh HY, et al. Hypoxia induces myeloid-derived suppressor cell recruitment to hepatocellular carcinoma through chemokine (C-C motif) ligand 26. *Hepatology.* (2016) 64:797–813. doi: 10.1002/hep.28655
161. Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z, et al. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J Immunol.* (2008) 181:346–53. doi: 10.4049/jimmunol.181.1.346
162. Wesolowski R, Markowitz J, Carson WE III. Myeloid derived suppressor cells—a new therapeutic target in the treatment of cancer. *J Immunother Cancer.* (2013) 1:10. doi: 10.1186/2051-1426-1-10
163. Alizadeh D, Larmonier N. Chemotherapeutic targeting of cancer-induced immunosuppressive cells. *Cancer Res.* (2014) 74:2663–8. doi: 10.1158/0008-5472.CAN-14-0301
164. Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic $Gr\text{-}1^+/CD11b^+$ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res.* (2005) 11:6713–21. doi: 10.1158/1078-0432.CCR-05-0883
165. Kuroda H, Mabuchi S, Kozasa K, Yokoi E, Matsumoto Y, Komura N, et al. PM01183 inhibits myeloid-derived suppressor cells *in vitro* and *in vivo*. *Immunotherapy.* (2017) 9:805–17. doi: 10.2217/imt-2017-0046
166. Mikyskova R, Indrova M, Vlkova V, Bieblova J, Simova J, Parackova Z, et al. DNA demethylating agent 5-azacytidine inhibits myeloid-derived suppressor cells induced by tumor growth and cyclophosphamide treatment. *J Leukoc Biol.* (2014) 95:743–53. doi: 10.1189/jlb.0813435
167. Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, Djeu JY. A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. *Clin Cancer Res.* (2010) 16:4583–94. doi: 10.1158/1078-0432.CCR-10-0733
168. Michels T, Shurin GV, Naiditch H, Sevko A, Umansky V, Shurin MR. Paclitaxel promotes differentiation of myeloid-derived suppressor cells into dendritic cells *in vitro* in a TLR4-independent manner. *J Immunotoxicol.* (2012) 9:292–300. doi: 10.3109/1547691X.2011.642418
169. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res.* (2010) 70:3052–61. doi: 10.1158/0008-5472.CAN-09-3690

170. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, et al. Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. *Cancer Res.* (2014) 74:104–18. doi: 10.1158/0008-5472.CAN-13-1545
171. Gujar SA, Clements D, Dielschneider R, Helson E, Marcato P, Lee PW. Gemcitabine enhances the efficacy of reovirus-based oncotherapy through anti-tumour immunological mechanisms. *Br J Cancer.* (2014) 110:83–93. doi: 10.1038/bjc.2013.695
172. Le HK, Graham L, Cha E, Morales JK, Manjili MH, Bear HD. Gemcitabine directly inhibits myeloid derived suppressor cells in BALB/c mice bearing 4T1 mammary carcinoma and augments expansion of T cells from tumor-bearing mice. *Int Immunopharmacol.* (2009) 9:900–9. doi: 10.1016/j.intimp.2009.03.015
173. Cao G, Wang J, Zheng X, Wei H, Tian Z, Sun R. Tumor Therapeutics Work as Stress Inducers to Enhance Tumor Sensitivity to Natural Killer (NK) Cell Cytolysis by Up-regulating NKp30 Ligand B7-H6. *J Biol Chem.* (2015) 290:29964–73. doi: 10.1074/jbc.M115.674010
174. Shi L, Lin H, Li G, Sun Y, Shen J, Xu J, et al. Cisplatin enhances NK cells immunotherapy efficacy to suppress HCC progression via altering the androgen receptor (AR)-ULBP2 signals. *Cancer Lett.* (2016) 373:45–56. doi: 10.1016/j.canlet.2016.01.017
175. Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. Markers of innate immune function are associated with vitamin a stores in men. *J Nutr.* (2009) 139:377–85. doi: 10.3945/jn.108.100198
176. Du Four S, Maenhout SK, De Pierre K, Renmans D, Niclou SP, Thielemans K, et al. Axitinib increases the infiltration of immune cells and reduces the suppressive capacity of monocytic MDSCs in an intracranial mouse melanoma model. *Oncoimmunology.* (2015) 4:e998107. doi: 10.1080/2162402X.2014.998107
177. Guislain A, Gadiot J, Kaiser A, Jordanova ES, Broeks A, Sanders J, et al. Sunitinib pretreatment improves tumor-infiltrating lymphocyte expansion by reduction in intratumoral content of myeloid-derived suppressor cells in human renal cell carcinoma. *Cancer Immunol Immunother.* (2015) 64:1241–50. doi: 10.1007/s00262-015-1735-z
178. Ko JS, Zea AH, Rini BI, Ireland JL, Elson P, Cohen P, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin Cancer Res.* (2009) 15:2148–57. doi: 10.1158/1078-0432.CCR-08-1332
179. Ozao-Choy J, Ma G, Kao J, Wang GX, Meseck M, Sung M, et al. The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. *Cancer Res.* (2009) 69:2514–22. doi: 10.1158/0008-5472.CAN-08-4709
180. Stiff A, Trikha P, Wesolowski R, Kendra K, Hsu V, Uppati S, et al. Myeloid-derived suppressor cells express Bruton's tyrosine kinase and can be depleted in tumor-bearing hosts by ibrutinib treatment. *Cancer Res.* (2016) 76:2125–36. doi: 10.1158/0008-5472.CAN-15-1490
181. Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer Res.* (2009) 69:2506–13. doi: 10.1158/0008-5472.CAN-08-4323
182. Steggerda SM, Bennett MK, Chen J, Emberley E, Huang T, Janes JR, et al. Inhibition of arginase by CB-1158 blocks myeloid cell-mediated immune suppression in the tumor microenvironment. *J Immunother Cancer.* (2017) 5:101. doi: 10.1186/s40425-017-0308-4
183. Huang Y, Wang Y, Li Y, Guo K, He Y. Role of sorafenib and sunitinib in the induction of expressions of NKG2D ligands in nasopharyngeal carcinoma with high expression of ABCG2. *J Cancer Res Clin Oncol.* (2011) 137:829–37. doi: 10.1007/s00432-010-0944-2
184. Xian P, Li Y, Zhou H, Luo H, Liu N, Dai JY. [Effect of Sunitinib therapy on immune function of patients with advanced renal cell carcinoma]. *Zhonghua Wai Ke Za Zhi.* (2016) 54:741–5. doi: 10.3760/cma.j.issn.0529-5815.2016.10.004
185. Wiers KM, Lathers DM, Wright MA, Young MR. Vitamin D3 treatment to diminish the levels of immune suppressive $CD34^+$ cells increases the effectiveness of adoptive immunotherapy. *J Immunother.* (2000) 23:115–24. doi: 10.1097/00002371-200001000-00014
186. Lathers DM, Clark JI, Achille NJ, Young MR. Phase 1B study to improve immune responses in head and neck cancer patients using escalating doses of 25-hydroxyvitamin D3. *Cancer Immunol Immunother.* (2004) 53:422–30. doi: 10.1007/s00262-003-0459-7
187. Mortara L, Gariboldi MB, Bosi A, Bregni M, Pinotti G, Guasti L, et al. Vitamin D deficiency has a negative impact on cetuximab-mediated cellular cytotoxicity against human colon carcinoma cells. *Target Oncol.* (2018) 13:657–65. doi: 10.1007/s11523-018-0586-x
188. Merrigan SL, Kennedy BN. Vitamin D receptor agonists regulate ocular developmental angiogenesis and modulate expression of dre-miR-21 and VEGF. *Br J Pharmacol.* (2017) 174:2636–51. doi: 10.1111/bph.13875
189. Lee GY, Han SN. The role of vitamin E in immunity. *Nutrients.* (2018) 10:10111614. doi: 10.3390/nu10111614
190. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res.* (2007) 67:11021–8. doi: 10.1158/0008-5472.CAN-07-2593
191. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, et al. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res.* (2006) 66:9299–307. doi: 10.1158/0008-5472.CAN-06-1690
192. Li Y, Wongsiriroj N, Blaner WS. The multifaceted nature of retinoid transport and metabolism. *Hepatobiliary Surg Nutr.* (2014) 3:126–39. doi: 10.3978/j.issn.2304-3881.2014.05.04
193. Iclozan C, Antonia S, Chiappori A, Chen DT, Gabrilovich D. Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer. *Cancer Immunol Immunother.* (2013) 62:909–18. doi: 10.1007/s00262-013-1396-8
194. Bauer R, Udonta F, Wroblewski M, Ben-Batalla I, Santos IM, Taverna F, et al. Blockade of myeloid-derived suppressor cell expansion with all-trans retinoic acid increases the efficacy of antiangiogenic therapy. *Cancer Res.* (2018) 78:3220–32. doi: 10.1158/0008-5472.CAN-17-3415
195. Li N, Lu Y, Li D, Zheng X, Lian J, Li S, et al. All-trans retinoic acid suppresses the angiopoietin-Tie2 pathway and inhibits angiogenesis and metastasis in esophageal squamous cell carcinoma. *PLoS ONE.* (2017) 12:e0174555. doi: 10.1371/journal.pone.0174555
196. Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, et al. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. *Int J Cancer.* (2003) 104:354–61. doi: 10.1002/ijc.10966
197. Li A, He M, Wang H, Qiao B, Chen P, Gu H, et al. All-trans retinoic acid negatively regulates cytotoxic activities of nature killer cell line 92. *Biochem Biophys Res Commun.* (2007) 352:42–7. doi: 10.1016/j.bbrc.2006.10.132
198. Konjevic G, Mirjacic-Martinovic K, Vuletic A, Babovic N. *In vitro* increased natural killer cell activity of metastatic melanoma patients with interferon-alpha alone as opposed to its combination with 13-cis retinoic acid is associated with modulation of NKG2D and CD161 activating receptor expression. *J BUON.* (2012) 17:761–9.
199. Blattner C, Fleming V, Weber R, Himmelhan B, Altevogt P, Gebhardt C, et al. $CCR5^+$ myeloid-derived suppressor cells are enriched and activated in melanoma lesions. *Cancer Res.* (2018) 78:157–67. doi: 10.1158/0008-5472.CAN-17-0348
200. Lin L, Deangelis S, Foust E, Fuchs J, Li C, Li PK, et al. A novel small molecule inhibits STAT3 phosphorylation and DNA binding activity and exhibits potent growth suppressive activity in human cancer cells. *Mol Cancer.* (2010) 9:217. doi: 10.1186/1476-4598-9-217
201. Bill MA, Fuchs JR, Li C, Yui J, Bakan C, Benson DM Jr., et al. The small molecule curcumin analog FLLL32 induces apoptosis in melanoma cells via STAT3 inhibition and retains the cellular response to cytokines with anti-tumor activity. *Mol Cancer.* (2010) 9:165. doi: 10.1186/1476-4598-9-165
202. Lu P, Yu B, Xu J. Cucurbitacin B regulates immature myeloid cell differentiation and enhances antitumor immunity in patients with lung cancer. *Cancer Biother Radiopharm.* (2012) 27:495–503. doi: 10.1089/cbr.2012.1219
203. Kortylewski M, Moreira D. Myeloid cells as a target for oligonucleotide therapeutics: turning obstacles into opportunities. *Cancer Immunol Immunother.* (2017) 66:979–88. doi: 10.1007/s00262-017-1966-2

204. Spinetti T, Spagnuolo L, Mottas I, Secondini C, Treinies M, Ruegg C, et al. TLR7-based cancer immunotherapy decreases intratumoral myeloid-derived suppressor cells and blocks their immunosuppressive function. *Oncoimmunology*. (2016) 5:e1230578. doi: 10.1080/2162402X.2016.1230578
205. Zhang Q, Hossain DM, Duttagupta P, Moreira D, Zhao X, Won H, et al. Serum-resistant CpG-STAT3 decoy for targeting survival and immune checkpoint signaling in acute myeloid leukemia. *Blood*. (2016) 127:1687–700. doi: 10.1182/blood-2015-08-665604
206. Orillion A, Hashimoto A, Damayanti N, Shen L, Adelaiye-Ogala R, Arisa S, et al. Entinostat neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of lung and renal cell carcinoma. *Clin Cancer Res.* (2017) 23:5187–201. doi: 10.1158/1078-0432.CCR-17-0741
207. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc Natl Acad Sci USA*. (2014) 111:11774–9. doi: 10.1073/pnas.1410626111
208. Hicks KC, Fantini M, Donahue RN, Schwab A, Knudson KM, Tritsch SR, et al. Epigenetic priming of both tumor and NK cells augments antibody-dependent cellular cytotoxicity elicited by the anti-PD-L1 antibody avelumab against multiple carcinoma cell types. *Oncoimmunology*. (2018) 7:e1466018. doi: 10.1080/2162402X.2018.1466018
209. Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W, et al. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J Exp Med.* (2006) 203:2691–702. doi: 10.1084/jem.20061104
210. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W, et al. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. *Proc Natl Acad Sci USA*. (2011) 108:17111–6. doi: 10.1073/pnas.1108121108
211. Lin S, Wang J, Wang L, Wen J, Guo Y, Qiao W, et al. Phosphodiesterase-5 inhibition suppresses colonic inflammation-induced tumorigenesis via blocking the recruitment of MDSC. *Am J Cancer Res.* (2017) 7:41–52.
212. Califano JA, Khan Z, Noonan KA, Rudraraju L, Zhang Z, Wang H, et al. Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:30–8. doi: 10.1158/1078-0432.CCR-14-1716
213. Weed DT, Vella JL, Reis IM, De la Fuente AC, Gomez C, Sargi Z, et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:39–48. doi: 10.1158/1078-0432.CCR-14-1711
214. Hassel JC, Jiang H, Bender C, Winkler J, Sevko A, Shevchenko I, et al. Tadalafil has biologic activity in human melanoma. Results of a pilot trial with Tadalafil in patients with metastatic Melanoma (TaMe). *Oncoimmunology*. (2017) 6:e1326440. doi: 10.1080/2162402X.2017.1326440
215. Hamilton TK, Hu N, Kolomitro K, Bell EN, Maurice DH, Graham CH, et al. Potential therapeutic applications of phosphodiesterase inhibition in prostate cancer. *World J Urol.* (2013) 31:325–30. doi: 10.1007/s00345-012-0848-7
216. Oberlies J, Watzl C, Giese T, Luckner C, Kropf P, Muller I, et al. Regulation of NK cell function by human granulocyte arginase. *J Immunol.* (2009) 182:5259–67. doi: 10.4049/jimmunol.0803523
217. Rodriguez PC, Ochoa AC, Al-Khami AA. Arginine metabolism in myeloid cells shapes innate and adaptive immunity. *Front Immunol.* (2017) 8:93. doi: 10.3389/fimmu.2017.00093
218. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res.* (2004) 64:5839–49. doi: 10.1158/0008-5472.CAN-04-0465
219. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature.* (2014) 513:559–63. doi: 10.1038/nature13490
220. Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *Immunol Rev.* (2008) 222:180–91. doi: 10.1111/j.1600-065X.2008.00608.x
221. Heys SD, Segar A, Payne S, Bruce DM, Kernohan N, Eremin O. Dietary supplementation with L-arginine: modulation of tumour-infiltrating lymphocytes in patients with colorectal cancer. *Br J Surg.* (1997) 84:238–41. doi: 10.1002/bjs.1800840229
222. Veltman JD, Lambers ME, van Nimwegen M, Hendriks RW, Hoogsteden HC, Aerts JG, et al. COX-2 inhibition improves immunotherapy and is associated with decreased numbers of myeloid-derived suppressor cells in mesothelioma. Celecoxib influences MDSC function. *BMC Cancer*. (2010) 10:464. doi: 10.1186/1471-2407-10-464
223. Kuroda H, Mabuchi S, Yokoi E, Komura N, Kozasa K, Matsumoto Y, et al. Prostaglandin E2 produced by myeloid-derived suppressive cells induces cancer stem cells in uterine cervical cancer. *Oncotarget.* (2018) 9:36317–30. doi: 10.18632/oncotarget.26347
224. Kosaka A, Ohkuri T, Okada H. Combination of an agonistic anti-CD40 monoclonal antibody and the COX-2 inhibitor celecoxib induces anti-glioma effects by promotion of type-1 immunity in myeloid cells and T-cells. *Cancer Immunol Immunother.* (2014) 63:847–57. doi: 10.1007/s00262-014-1561-8
225. Iachininoto MG, Nuzzolo ER, Bonanno G, Mariotti A, Procoli A, Locatelli F, et al. Cyclooxygenase-2 (COX-2) inhibition constrains indoleamine 2,3-dioxygenase 1 (IDO1) activity in acute myeloid leukaemia cells. *Molecules.* (2013) 18:10132–45. doi: 10.3390/molecules180910132
226. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol.* (2009) 182:5693–701. doi: 10.4049/jimmunol.0900092
227. Kim SJ, Ha GH, Bae JH, Kim GR, Son CH, Park YS, et al. COX-2- and endoplasmic reticulum stress-independent induction of ULBP-1 and enhancement of sensitivity to NK cell-mediated cytotoxicity by celecoxib in colon cancer cells. *Exp Cell Res.* (2015) 330:451–9. doi: 10.1016/j.yexcr.2014.09.008
228. Majumder M, Nandi P, Omar A, Ugwuagbo KC, Lala PK. EP4 as a therapeutic target for aggressive human breast cancer. *Int J Mol Sci.* (2018) 19:19041019. doi: 10.3390/ijms19041019
229. Ohl K, Tenbrock K. Reactive oxygen species as regulators of MDSC-mediated immune suppression. *Front Immunol.* (2018) 9:2499. doi: 10.3389/fimmu.2018.02499
230. Nagaraj S, Youn JI, Weber H, Iclozan C, Lu L, Cotter MJ, et al. Anti-inflammatory triterpenoid blocks immune suppressive function of MDSCs and improves immune response in cancer. *Clin Cancer Res.* (2010) 16:1812–23. doi: 10.1158/1078-0432.CCR-09-3272
231. Wang YY, Yang YX, Zhe H, He ZX, Zhou SF. Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. *Drug Des Devel Ther.* (2014) 8:2075–88. doi: 10.2147/DDDT.S68872
232. O'Sullivan T, Saddawi-Konefka R, Gross E, Tran M, Mayfield SP, Ikeda H, et al. Interleukin-17D mediates tumor rejection through recruitment of natural killer cells. *Cell Rep.* (2014) 7:989–98. doi: 10.1016/j.celrep.2014.03.073
233. Saddawi-Konefka R, O'Sullivan T, Gross ET, Washington A Jr., Bui JD. Tumor-expressed IL-17D recruits NK cells to reject tumors. *Oncoimmunology.* (2014) 3:e954853. doi: 10.4161/21624011.2014.954853
234. Saddawi-Konefka R, Seelige R, Gross ET, Levy E, Searles SC, Washington A Jr., et al. Nrf2 induces IL-17D to mediate tumor and virus surveillance. *Cell Rep.* (2016) 16:2348–58. doi: 10.1016/j.celrep.2016.07.075
235. Seelige R, Saddawi-Konefka R, Adams NM, Picarda G, Sun JC, Benedict CA, et al. Interleukin-17D and Nrf2 mediate initial innate immune cell recruitment and restrict MCMV infection. *Sci Rep.* (2018) 8:13670. doi: 10.1038/s41598-018-32011-2
236. Kim SH, Li M, Trousil S, Zhang Y, Pasca di Magliano M, Swanson KD, et al. Phenformin inhibits myeloid-derived suppressor cells and enhances the anti-tumor activity of PD-1 blockade in melanoma. *J Invest Dermatol.* (2017) 137:1740–8. doi: 10.1016/j.jid.2017.03.033
237. Qin G, Lian J, Huang L, Zhao Q, Liu S, Zhang Z, et al. Metformin blocks myeloid-derived suppressor cell accumulation through AMPK-DACH1-CXCL1 axis. *Oncoimmunology.* (2018) 7:e1442167. doi: 10.1080/2162402X.2018.1442167

238. Uehara T, Eikawa S, Nishida M, Kunisada Y, Yoshida A, Fujiwara T, et al. Metformin induces CD11b^{+} cell-mediated growth inhibition of an osteosarcoma: implications for metabolic reprogramming of myeloid cells and antitumor effects. *Int Immunol.* (2018) 31:187–98. doi: 10.1093/intimm/dxy079
239. de Mey S, Jiang H, Wang H, Engels B, Gevaert T, Dufait I, et al. Potential of memory T cells in bridging preoperative chemoradiation and immunotherapy in rectal cancer. *Radiother Oncol.* (2018) 127:361–9. doi: 10.1016/j.radonc.2018.04.003
240. Li L, Wang L, Li J, Fan Z, Yang L, Zhang Z, et al. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* (2018) 78:1779–91. doi: 10.1158/0008-5472.CAN-17-2460
241. Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. *Cancer Immunol Res.* (2017) 5:9–16. doi: 10.1158/2326-6066.CIR-16-0103
242. Kannarkatt J, Alkharabsheh O, Tokala H, Dimitrov NV. Metformin and angiogenesis in cancer—revisited. *Oncology.* (2016) 91:179–84. doi: 10.1159/000448175
243. Orecchioni S, Reggiani F, Talarico G, Mancuso P, Calleri A, Gregato G, et al. The biguanides metformin and phenformin inhibit angiogenesis, local and metastatic growth of breast cancer by targeting both neoplastic and microenvironment cells. *Int J Cancer.* (2015) 136:E534–544. doi: 10.1002/ijc.29193
244. Dallaglio K, Bruno A, Cantelmo AR, Esposito AI, Ruggiero L, Orecchioni S, et al. Paradoxic effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis.* (2014) 35:1055–66. doi: 10.1093/carcin/bgu001
245. Cha JH, Yang WH, Xia W, Wei Y, Chan LC, Lim SO, et al. Metformin promotes antitumor immunity via endoplasmic-reticulum-associated degradation of PD-L1. *Mol Cell.* (2018) 71:606–20.e607. doi: 10.1016/j.molcel.2018.07.030
246. Pereira FV, Melo ACL, Low JS, de Castro IA, Braga TT, Almeida DC, et al. Metformin exerts antitumor activity via inductionof multiple death pathways in tumor cells and activation of a protective immune response. *Oncotarget.* (2018) 9:25808–25. doi: 10.18632/oncotarget.25380
247. Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. *Proc Natl Acad Sci USA.* (2015) 112:1809–14. doi: 10.1073/pnas.1417636112
248. Ho V, Lim TS, Lee J, Steinberg J, Szmyd R, Tham M, et al. TLR3 agonist and Sorafenib combinatorial therapy promotes immune activation and controls hepatocellular carcinoma progression. *Oncotarget.* (2015) 6:27252–66. doi: 10.18632/oncotarget.4583
249. Seya T, Shime H, Matsumoto M. Functional alteration of tumor-infiltrating myeloid cells in RNA adjuvant therapy. *Anticancer Res.* (2015) 35:4385–92.
250. Shime H, Kojima A, Maruyama A, Saito Y, Oshiumi H, Matsumoto M, et al. Myeloid-derived suppressor cells confer tumor-suppressive functions on natural killer cells via polyinosinic:polycytidylic acid treatment in mouse tumor models. *J Innate Immun.* (2014) 6:293–305. doi: 10.1159/000355126
251. Pang X, Song H, Zhang Q, Tu Z, Niu J. Hepatitis C virus regulates the production of monocytic myeloid-derived suppressor cells from peripheral blood mononuclear cells through PI3K pathway and autocrine signaling. *Clin Immunol.* (2016) 164:57–64. doi: 10.1016/j.clim.2016.01.014

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Myeloid-Derived Suppressor Cells in Lung Transplantation

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Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of immune cells from the myeloid lineage. MDSCs expand in pathological situations, such as chronic infection, cancer, autoimmunity, and allograft rejection. As chronic lung allograft dysfunction (CLAD) limits long-term survival after lung transplantation (LTx), MDSCs may play a role in its pathophysiology. We assessed phenotype and frequency of MDSCs in peripheral blood from lung transplant recipients and its relationship to post-transplant complications and immunosuppression. Granulocytic (G)-MDSC were identified and quantified by flow cytometry of blood from 4 control subjects and 20 lung transplant patients (stable $n = 6$, infection $n = 5$; CLAD $n = 9$). G-MDSC functionality was assessed *in vitro* by their capability to block CD4 and CD8 T cell proliferation. More G-MDSC could be assessed using EDTA tubes compared to heparin tubes ($p = 0.004$). G-MDSC were increased in stable lung transplant recipients vs. non-transplant controls (52.1% vs. 9.4%; $p = 0.0095$). The infection or CLAD groups had lower G-MDSCs vs. stable recipients (28.2%$p = 0.041$ and 33.0%; $p = 0.088$, respectively), but were not different among CLAD phenotypes. G-MDSC tended to correlate with cyclosporine A and tacrolimus levels ($r^2 = 0.18$; $r^2 = 0.17$). CD4 and CD8 cells proliferation decreased by 50 and 80% if co-cultured with MDSCs (1:6 and 1:2 MDSC:T-cell ratio, respectively). In conclusion, circulating MDSCs are measurable, functional and have a G-MDSC phenotype in lung transplant patients. Their frequency is increased in stable patients, decreased during post-transplant complications, and related to level of immunosuppression. This study may pave the way for further investigations of MDSC in the context of lung transplantation.

Keywords: myeloid-derived suppressor cells, blood, lung transplantation, allograft, chronic rejection, immunosuppression, infection, phenotypes

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INTRODUCTION

From a transplant immunological point of view, graft acceptance is the fundamental element in allograft survival. Graft acceptance is realized by blocking the immune system with immunosuppression preventing host immune cells to recognized and attack the "non-self" donor (lung) tissue. Immune regulatory cells are thought to play a major role in the balance between graft acceptance and chronic rejection. Most attention has gone to natural and inducible FoxP3 positive

regulatory T cells (Treg) (1). Immune regulation and graft acceptance, however, encompasses many more cells including regulatory B cells, regulatory dendritic cells and innate regulatory cells like the myeloid-derived suppressor cells (MDSCs), which were introduced 10 years ago by Gabrilovich et al., MDSCs were initially described as a heterogeneous group of immune cells from the myeloid lineage with a potent immune-regulatory activity (2). In the last few years, more insights into the nature and biological role of MDSCs have been reported and consequently MDSCs have emerged as a universal regulator of immune function in many pathologic conditions. MDSCs are known to expand in pathological situations such as chronic infection, cancer, transplant rejection and autoimmunity (3–5). Within the MDSC population, two main subgroups of cells were identified: granulocytic MDSCs (G-MDSCs) also nominated as polymorphomononuclear (PMN-MDSCs) and monocytic (M)-MDSCs. G-MDSCs are phenotypically and morphologically similar to neutrophils, whereas M-MDSCs resemble monocytes (6). Looking at the functionality of both M- and G-MDCS, the suppressive activity has been mainly attributed to arginine 1 (ARG1) and nitric oxide (NO) for M-MDSC and upregulation of reactive oxygen species (ROS) for G-MDSC (7, 8). Upregulation of ARG1, NO, and ROS are key mechanism to suppress T cell proliferation (9) and the production of IFNγ (10). Another hallmark is the upregulation of the transcription factor signal transducer and activator of transcription 3 (STAT3). STAT3, which functions as a signaling hub, integrating the different cues of the immunologic micro-environment (11, 12) regulates the expansion of MDSCs by stimulating myelopoiesis and inhibiting myeloid-cell differentiation. Further, it promotes MDSC survival by inducing the expression of cyclin D1, B-cell lymphoma XL (BCL-XL) and MYC (4). Within transplantation, MDSCs are involved in maintaining allogeneic acceptance in bone marrow, kidney and liver transplantation (13–16). Moreover, it has also been shown that commonly used immunosuppressive drugs can affect MDSC differentiation and functionality (17, 18). Our goal was to characterize phenotype (M-MDSC or G-MDSC) and frequency of MDSCs in lung transplant recipients. And consequently, to assess if MDSCs can serve as a potential new research target in the field of lung transplantation since chronic lung allograft dysfunction (CLAD), considered to be driven by an overactive T cell response,

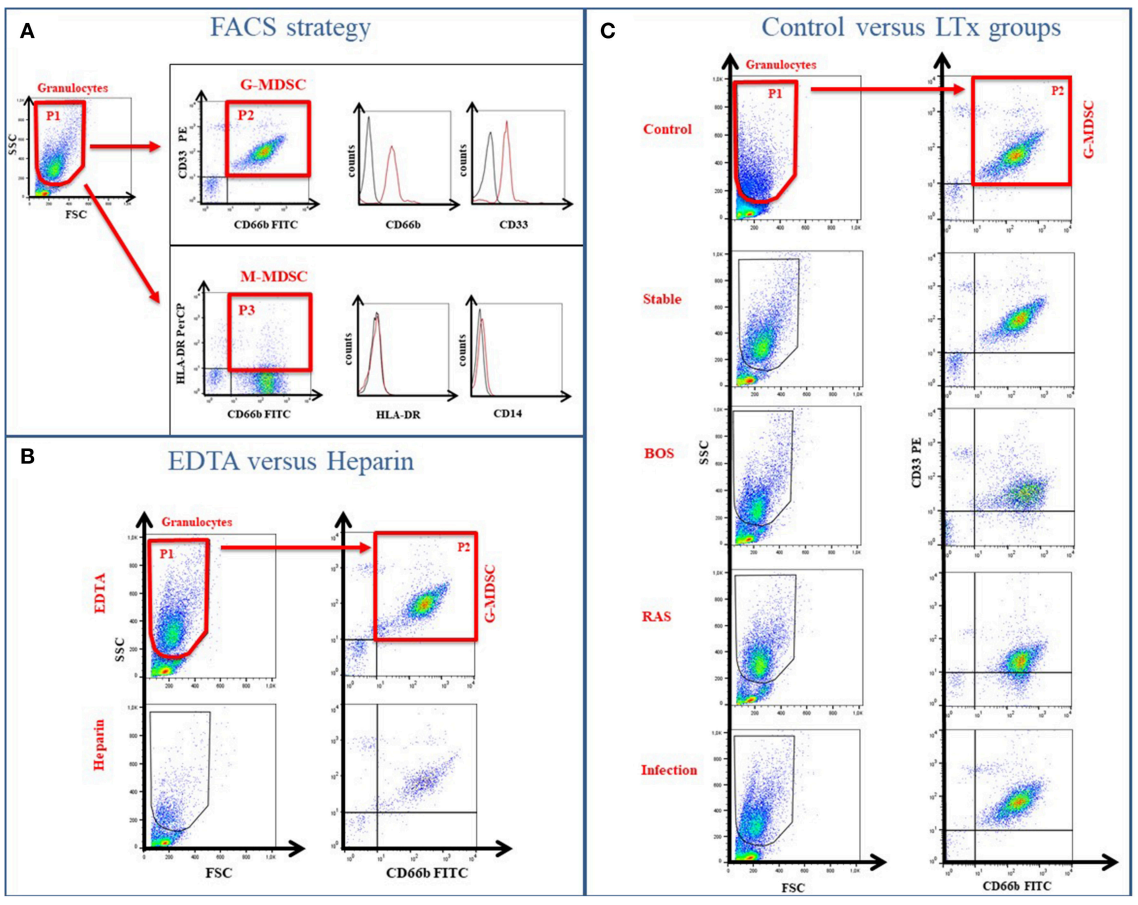

FIGURE 1 | Gating Strategy to determine MDSC phenotype. **(A)** The low-density fraction of PBMC was stained with specific markers to differentiate between G-MDSCs (CD66b/CD33) and M-MDSCs (HLA-DR/CD14). **(B)** Different coatings of blood tubes (EDTA vs. Heparin) affect the MDSC cell numbers. **(C)** Exemplary FACS plots of the healthy controls and different LTx patient groups.

remains the most important factor limiting long-term survival after transplantation.

METHODS

Patient Characteristics

This study included 20 lung transplant recipients and 4 healthy controls recruited at the University Hospitals Leuven (Belgium). All lung transplant recipients gave informed consent at time of listing for transplantation and routine blood sampling was approved by the University hospital (S51577). Relevant patient information retrieved from the clinical database included age, gender, type of transplantation, underlying disease, allograft ischemic time during transplantation, immunosuppressive dose, and trough levels, time post-transplant of blood sampling, time of death, infection information, and diagnostic criteria for CLAD and its phenotypes. Lung transplant recipients were selected according to their clinical status upon recruitment: 6 were considered stable, 5 recipients had an acute infection (2 CMV; 1 *Pseudomonas aeruginosa*; 1 Influenza + *E. coli*; 1 Influenza + *Aspergillus fumigatus*) and 9 were affected by different phenotypes of CLAD (5 BOS and 4 RAS cases). Blood of 15 individuals was used to compare Heparin vs. EDTA coated blood tubes (2 control, 3 Infection, 5 Stable, and 5 CLAD). The clinical status was assessed by an expert clinician (RV) according to current guidelines (19, 20).

MDSC Characterization

Peripheral blood was collected using EDTA and Heparin-coated tubes and samples were shipped to the Universitätsklinik für Kinder-und Jugendmedizin, Tübingen (Germany) at room temperature and analyzed within 24 h. MDSCs were

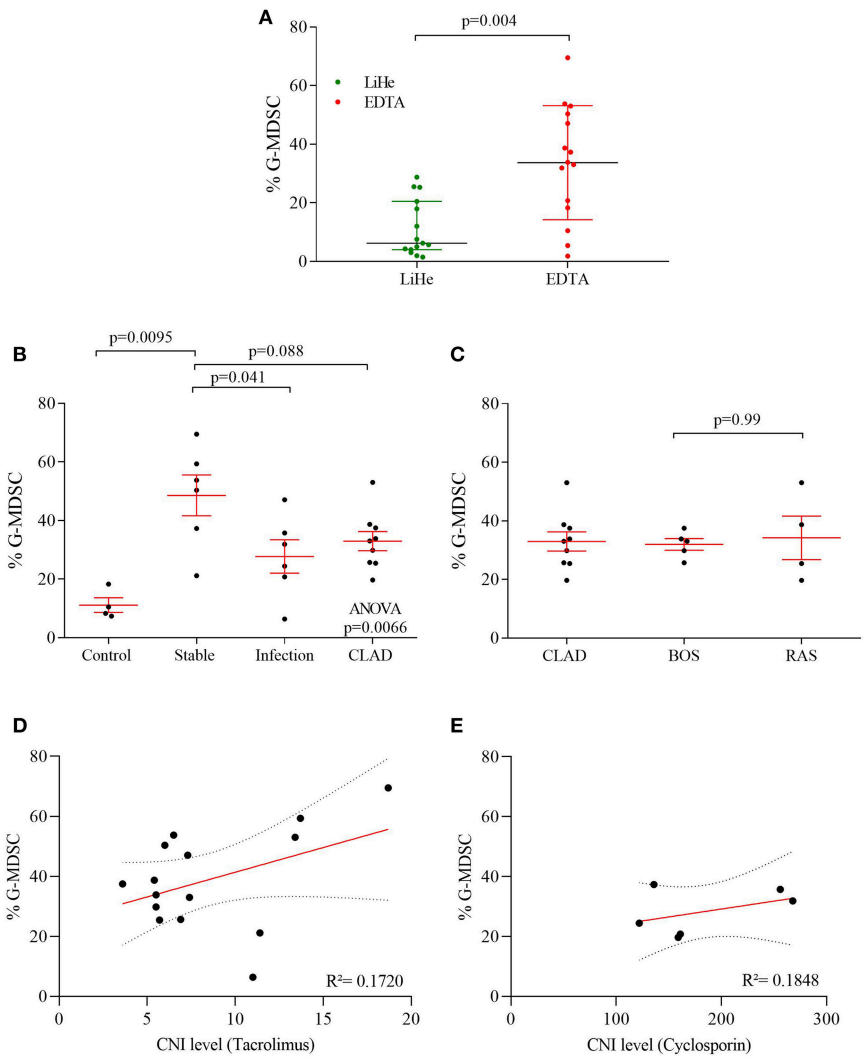

FIGURE 2 | G-MDSC percentages measured in blood of lung transplant recipients and healthy controls. **(A)** the effect of LiHe vs. EDTA tubes on G-MDSC percentages; **(B)** G-MDSC in healthy controls and lung transplant recipients who were stable, had an infection or were diagnosed with CLAD; **(C)** CLAD sub-phenotypes in BOS and RAS. **(D,E)** G-MDSC correlated with CNI level of the patients.

characterized as previously described (21, 22). In brief, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll density gradient centrifugation (Lymphocyte Separation Medium; Biochrom), washed with RPMI-1640 and cell viability was confirmed by trypan blue staining. The isolated PBMC, containing only low density granulocytes, were stained with specific antibodies for G-MDSC (CD66b-FITC, CD33-PE) and M-MDSC (CD14-FITC and HLADR-PerCP) (Miltenyi Biotec) and quantified by flow cytometry using a FACSCalibur (BD). G-MDSCs were phenotypically characterized as low-density fraction granulocytes $CD33^+CD66b^+$ cells (**Figure 1**). The percentage of G-MDSC was determined as ratio of $CD33^+CD66b^+$ cells (P2 in **Figure 1**) over total PBMCs containing the low density granulocyte fraction (P1 in **Figure 1**). Calculations were performed with BD CellQuest Pro analysis software and FlowJo V7.

T-Cell Suppression Assays

The MDSC functional assay assessed T-cell suppression (both CD4 and CD8) by isolated MDSC (**Figure 2**) (23). MDSCs were isolated from blood of 2 lung transplant recipients, 1 stable and 1 with CLAD (BOS), using anti-CD66b and anti-FITC magnetic microbeads with the autoMACS® Pro Separator (Miltenyi Biotec) according to manufacturer's instructions. $CD4^+$ and $CD8^+$ T cells were isolated using CD4 and CD8 antibody (BD Pharmingen) combined with anti-FITC magnetic microbeads and autoMACS® Pro Separator (Miltenyi Biotec). Isolated CD4 or CD8 cells were labeled with CFSE dissolved in RPMI-1640, supplemented with 10% heat-inactivated human serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin and 60,000 cells were plated per well in a 96-well microtiter plate. Cells were further stimulated with 100 U/ml IL-2 (R&D Systems) and 1 μg/ml OKT3 (Janssen Cilag). Different numbers of G-MDSCs were added to obtain an MDSC:T-cell ratio 1:6 and 1:2 and incubated for 3 days in a humidified chamber at 37°C and 5% CO_2. After incubation, cells were harvested and CFSE-fluorescence intensity analyzed by flow cytometry to determine T-cell proliferation. Proliferation was calculated as the ratio of the divided cells (P1 to P5) over all cells (P0 to P5) with control T cells as reference value.

Statistical Analysis

Qualitative variables are expressed as absolute numbers and percentages. Normally distributed quantitative variables are expressed as mean and standard deviation; non-normally distributed variables are expressed as median and interquartile range (25–75 percentile). Demographic and clinical variables of patients were compared using the chi-square test for qualitative variables or Fisher's exact test when one of the expected effects was <5. Normally distributed quantitative variables were compared using one-way ANOVA test; non-normally distributed quantitative variables were compared using the Kruskal-Wallis test. One-way ANOVA Test was used to compare MDSCs counts between groups. Linear regression was used for investigating the interaction of MDSC% and immunosuppressive trough levels. Data were analyzed using Graph Pad prism 7.0 software (San Diego, CA, USA).

TABLE 1 | Characteristics of lung transplant patients.

	All	Stable	Infection	CLAD	*p*
	n = 20	*n* = 6	*n* = 5	*n* = 9	
Age, median (IQR)	55 (32–60)	51.5 (20–60)	58 (36–62)	54 (39–57)	0.65
Gender: Male, *n* (%)	9 (42.9)	2 (33.3)	2 (40.0)	4 (44.4)	0.17
Diagnosis, *n* (%)					
COPD	10 (50.0)	3 (50.0)	3 (60.0)	4 (44.4)	0.86
ILD	2 (10.0)	0 (0.0)	0 (0.0)	2 (22.2)	0.47
CF	5 (25.0)	2 (33.3)	1 (20.0)	2 (22.2)	1.00
Other	3 (15.0)	1 (16.7)	1 (20.0)	1 (11.1)	1.00
Immunosupressive treatment, *n* (%)					
CsA+AZA+P	2 (10.0)	1 (16.7)	0 (0.0)	1 (11.1)	1.00
CsA+MMF+P	2 (10.0)	0 (0.0)	2 (40.0)	0(0.0)	0.05
CsA+P	1 (5.0)	0 (0.0)	1 (20.0)	0 (0.0)	0.25
FK+AZA+P	5 (25.0)	1 (16.7)	1 (20.0)	3 (33.3)	0.82
FK+MMF+P	5 (25.0)	3 (50.0)	1 (20.0)	1 (11.1)	0.35
FK+P	4 (20.0)	1 (16.7)	0 (0.0)	3 (33.3)	0.41
FK	1 (5.0)	0 (0.0)	0 (0.0)	1 (11.1)	1.00
Type of LTx					
SSLT	18 (90.0)	6 (100.0)	5 (100.0)	7 (77.8)	0.48
SLT	2 (10.0)	0 (0.0)	0 (0.0)	2 (22.2)	
Survival post LTx (years), median (IQR)	7.0 (4.1–9.7)	5.6 (3.8–8.7)	4.3 (3.0–7.4)	7.5 (5.9–11.9)	0.18
Sampling time post LTx (months), median (IQR)	3.9 (0.9–6.6)	1.9 (0.7–5.1)	0.9 (0.5–4.7)	6.6 (4.8–9.3)	0.02

IQR, interquartile range; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; CF, cystic fibrosis; CsA, Cyclosporine; FK, Tacrolimus; P, Prednisolone; LTx, Lung Transplantation.

RESULTS

Clinical characteristics of study participants are included in **Table 1**.

G-MDSC were present in the low-density fraction of PBMCs, based on physical (FSC/SSC) and flow cytometric characteristics ($CD33^+CD66b^+$ cells) (**Figure 1A**). M-MDSC, on the other hand, were not observed in the low-density fraction of PBMCs, based on physical (FSC/SSC) and cell surface marker characteristics ($CD14^-$HLA-DR^-) (**Figure 1A**).

Percentages of G-MDSC were increased when using EDTA tubes compared to using LiHe tubes (mean: 33.38% [range: 18.32–50.36] vs. 6.24% [4.02–20.53], $p = 0.004$) (**Figures 1B**, **2A**). EDTA and LiHe tubes were equally (statistically not significantly different) distributed across the control and patient groups. G-MDSC were increased in stable lung transplant recipients vs. healthy control subjects (52.1% [33.3–61.9] vs. 9.4% [7.6–16.4], $p = 0.0095$) (**Figures 1C**, **2B**). Lung transplant recipients with an infection or CLAD tended to have lower percentage of G-MDSC compared to stable recipients (28.2% [17.2–36.6], $p = 0.041$ and 33.0% [25.6-38.1], $p = 0.088$, respectively) (**Figure 2B**). Within CLAD patients, the proportion of G-MDSC were comparable in BOS (5 cases) and RAS (4 cases) ($p = 0.99$) (**Figure 2C**). G-MDSC percentages seemed to increase with increasing blood levels

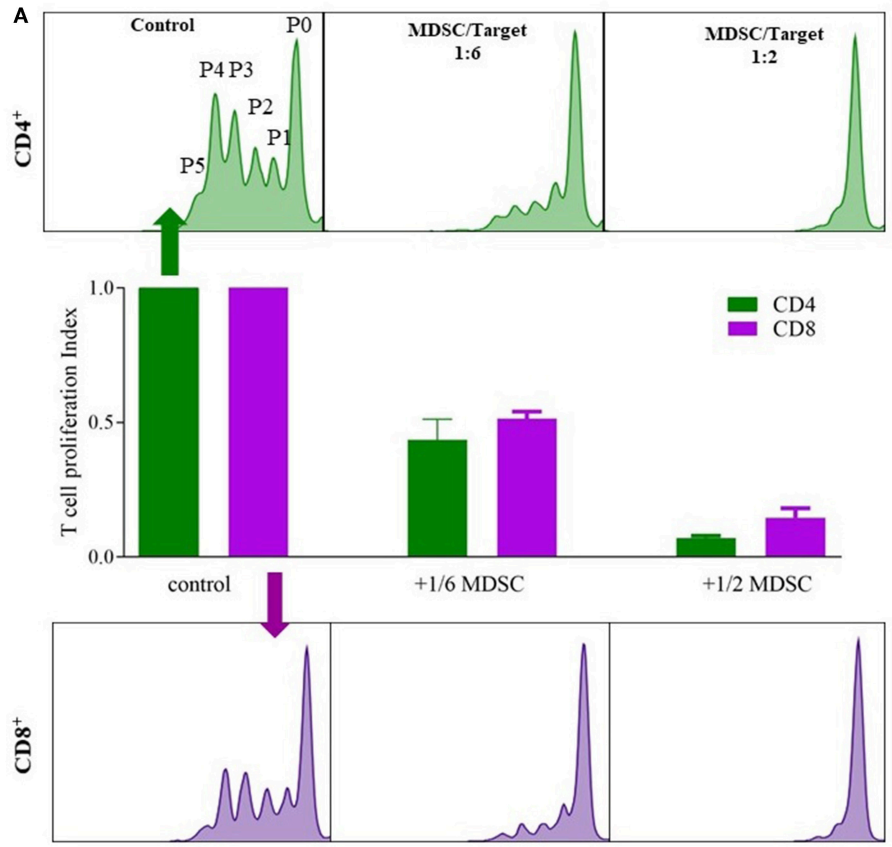

FIGURE 3 | G-MDSCs isolated from lung transplant patients functionally supress T cell proliferation. The suppressive effect of CD66b$^+$-MACS-isolated MDSCs (isolated from lung transplant recipients; 1 with CLAD and 1 stable) on CFSE labeled T cell CD4$^+$ (green) and CD8$^+$ (purple) proliferation. **(A)** Different ratios of MDSC vs. T cells (1:6 and 1:2) were assessed and compared with T cell proliferation without MDSCs. P0 represents undivided cells, P1 cells divided 1 time; P2 cells divided twice and so on. T cell proliferation ratio is portion of divided cells over all cells. The bar graphs represent the proliferation index compared to control conditions ($n = 2$).

of the calcineurin inhibitors (Tacrolimus $r^2 = 0.17$, $p = 0.12$; Cyclosporine $r^2 = 0.18$, $p = 0.39$) used as immunosuppressive therapy, which however was not significant most probably due to the small sample size (**Figures 2D,E**).

G-MDSCs isolated from lung transplant patients effectively suppressed T-cell proliferation in a CFSE based polyclonal proliferation assay. The T-cell suppression assay was used as a proof-of-concept assay to demonstrate that G-MDSCs expanded in transplant recipient patients indeed represent a suppressive G-MDSC cell type and do not reflect myeloid cell populations with G-MDSC-like markers, but without T cell suppressive activities. Isolated patient G-MDSCs exhibited a strong suppressive function on T cell proliferation of about 50 and 80% with a 1:6 and 1:2 ratio of MDSC, vs. CD4$^+$ or CD8$^+$ T cells, respectively (**Figure 3**).

DISCUSSION

MDSCs were evaluated in lung transplant recipients and G-MDSC (CD33$^+$/CD66b$^+$) could be identified in the low-density fraction of PBMCs. G-MDSC (CD33$^+$/CD66b$^+$) cells also expressed CD11b, CXCR4 and HLA-DR^{low}. The absence of CD14 expression confirmed their G-MDSC phenotype (**Figure S1**).

MDSCs are known for their role in immune regulation and allograft acceptance, and are involved in delayed graft rejection (17, 24, 25). Our data showed an expansion of G-MDSCs (not M-MDSCs) in stable lung transplant recipients and a decrease of G-MDSCs in patients with CLAD. Lung transplant recipients suffering from an infection also demonstrated a reduction in G-MDSCs, pointing to the fact that infection interferes with immune regulation and allograft acceptance. For example, it has been shown in mice that CMV infection impairs MDSC differentiation (26). CMV is a clinically relevant post-transplant pathogen, which is considered as a risk factor for later development of CLAD (27) Also in our study population, we found that recipients with diagnosed CMV within the infection group showed a lower G-MDSC percentage compared to the other patient groups (data not shown).

Furthermore, we evaluated the effect of immunosuppression on G-MDSCs: G-MDSCs showed a modest correlation with increasing CNI trough levels, a previously reported phenomenon

(17, 28). Calcineurin inhibitors are indispensable in lung transplantation as efficient immunosuppressive drugs to block the immune response toward the allograft; hence, induction of MDSCs and their immunosuppressive function might be a part of their mechanism of action. It has been shown in a mouse skin transplant model that mechanistically, CsA treatment enhances the expression of indoleamine 2,3-dioxygenase (IDO) and thereby induces the suppressive activities of MDSCs in allograft recipients (29). Since the myeloid compartment consists of many different cell types with often overlapping phenotypic markers, we wanted to assess if the G-MDSCs, isolated from our lung transplant population, demonstrated suppressive effector properties. We confirmed that G-MDSCs did exert $CD4^+$ and $CD8^+$ T cell suppression in two independent patient samples. Due to the low number of replications, we can only speculate that in the setting of transplant immunology, G-MDSCs would act upstream of T cells to induce a cascade of peripheral tolerance toward the graft tissue. Challenging from a technical standpoint was the difference observed between the Lithium-Heparin and EDTA coated blood-drawing tubes used for PBMC isolations and the resulting differences in G-MDSC. At this point, we speculate that EDTA, as an iron chelator, inhibits cell degranulation, and may be the reason why more G-MDSC can be measured when using EDTA coating compared to Lithium-Heparin, at least in our experimental settings. However, it is important to mention that in a study by Pallet et al., the opposite effect, increased G-MDSC counts in Heparin vs. EDTA tubes, has been observed (30), which thus needs further investigation.

There are several limitations to our study. As a pilot study, the number of studied patients is limited. Furthermore, there are several confounding factors such as the heterogeneity of patient characteristics, differences in immunosuppressive therapy, use of azithromycin, different blood sampling tubes and different timings of sampling after lung transplantation.

However, our findings remain interesting, and may warrant more in-depth research on the role of G-MDSCs in lung transplantation. In our opinion, elucidating the functional hierarchy of immune regulatory cells in the context of transplant tolerance/rejection is of importance to understand graft acceptance. We believe that the up-stream suppressive activity of G-MDSC may be an intriguing starting point to dissect this highly complex interconnected immune regulatory system consisting of Treg, Bregs, Mregs, and other cell types.

ETHICS STATEMENT

This study included 20 lung transplant recipients and 4 healthy controls recruited at the University Hospitals Leuven (Belgium). All lung transplant recipients gave informed consent at time of listing for transplantation and routine blood sampling was approved by the University hospital (S51577).

AUTHOR CONTRIBUTIONS

TH and ASi performed this study from sampling, analyzing, writing, and submitting the paper. ASi performed the MDSC FACS analysis, the *in vitro* MDSC proliferation test and the writing of the paper. JK, ASa, and SV helped in organizing the sampling and writing of the paper. DH helped in writing of the paper. BS-G, HB, AV, and AVH helped by searching for the clinical patient information and helped preparing the data. DV was the surgeon performing the lung translations and critical evaluated the manuscript. GV and RV were the clinicians performing the daily care taking of the patients and critical evaluated the manuscript. BV and RV designed and funded the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00900/full#supplementary-material

Figure S1 | Representative dot plots and histograms of surface marker profiling of G-MDSCs isolated from lung transplant patients. MDSCs were analyzed in peripheral blood mononuclear cells (PBMCs) isolated from low density fraction of whole blood after Ficoll density centrifugation. Lung transplant MDSCs exhibited characteristic G-MDSC phenotype of $CD33b^+CD14^-$ cells (**left** panel, also see **Figure 1**) and also expressed CXCR4 (**right** panel). Histograms show individual surface marker staining (red) in comparison to unstained control (black) for CXCR4.

REFERENCES

1. Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. *Nat Rev Immunol.* (2012) 12:417–30. doi: 10.1038/nri3227
2. Gabrilovich DI, Bronte V, Chen S-H, Colombo MP, Ochoa A, Ostrand-Rosenberg S, et al. The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* (2007) 67:425. doi: 10.1158/0008-5472.CAN-06-3037
3. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol.* (2009) 182:4499–506. doi: 10.4049/jimmunol.0802740
4. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* (2009) 9:162–74. doi: 10.1038/nri2506
5. Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J Immunol.* (2006) 176:284–90. doi: 10.4049/jimmunol.176.1.284
6. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* (2012) 12:253–68. doi: 10.1038/nri3175
7. Youn J-I, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in

tumor-bearing mice. *J Leukoc Biol*. (2012) 91:167–81. doi: 10.1189/jlb.0311177
8. Umansky V, Blattner C, Gebhardt C, Utikal J. The Role of Myeloid-Derived Suppressor Cells (MDSC) in cancer progression. *Vaccines*. (2016) 4:E36. doi: 10.3390/vaccines4040036
9. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J Exp Med*. (2005) 202:931–9. doi: 10.1084/jem.20050715
10. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol*. (2013) 13:349–61. doi: 10.1038/nri3423
11. Nefedova Y, Huang M, Kusmartsev S, Bhattacharya R, Cheng P, Salup R, et al. Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer. *J Immunol*. (2004) 172:464–74. doi: 10.4049/jimmunol.172.1.464
12. Rébé C, Végran F, Berger H, Ghiringhelli F. STAT3 activation: a key factor in tumor immunoescape. *JAKSTAT*. (2013) 2:e23010. doi: 10.4161/jkst.23010
13. Highfill SL, Rodriguez PC, Zhou Q, Goetz CA, Koehn BH, Veenstra R, et al. Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13. *Blood*. (2010) 116:5738–47. doi: 10.1182/blood-2010-06-287839
14. Meng F, Chen S, Guo X, Chen Z, Huang X, Lai Y, et al. Clinical significance of myeloid-derived suppressor cells in human renal transplantation with acute T cell-mediated rejection. *Inflammation*. (2014) 37:1799–805. doi: 10.1007/s10753-014-9910-5
15. Luan Y, Mosheir E, Menon MC, Wilson D, Woytovich C, Ochando J, et al. Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion. *Am J Transplant*. (2013) 13:3123–31. doi: 10.1111/ajt.12461
16. Natarajan S, Thomson AW. Tolerogenic dendritic cells and myeloid-derived suppressor cells: potential for regulation and therapy of liver auto- and alloimmunity. *Immunobiology*. (2010) 215:698–703. doi: 10.1016/j.imbio.2010.05.024
17. Han C, Wu T, Na N, Zhao Y, Li W, Zhao Y. The effect of immunosuppressive drug cyclosporine A on myeloid-derived suppressor cells in transplanted mice. *Inflamm Res*. (2016) 65:679–88. doi: 10.1007/s00011-016-0949-7
18. Okano S, Abu-Elmagd K, Kish DD, Keslar K, Baldwin WM, Fairchild RL, et al. Myeloid-derived suppressor cells increase and inhibit donor-reactive T cell responses to graft intestinal epithelium in intestinal transplant patients. *Am J Transplant*. (2018) 18:2544–58. doi: 10.1111/ajt.14718
19. Meyer KC, Raghu G, Verleden GM, Corris PA, Aurora P, Wilson KC, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. *Eur Respir J*. (2014) 44:1479–503. doi: 10.1183/09031936.00107514
20. Verleden GM, Raghu G, Meyer KC, Glanville AR, Corris P. A new classification system for chronic lung allograft dysfunction. *J Heart Lung Transplant*. (2014) 33:127–33. doi: 10.1016/j.healun.2013.10.022
21. Brandau S, Trellakis S, Bruderek K, Schmaltz D, Steller G, Elian M, et al. Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. *J Leukoc Biol*. (2011) 89:311–7. doi: 10.1189/jlb.0310162
22. Rieber N, Brand A, Hector A, Graepler-Mainka U, Ost M, Schäfer I, et al. Flagellin induces myeloid-derived suppressor cells: implications for pseudomonas aeruginosa infection in cystic fibrosis lung disease. *J Immunol*. (2013) 190:1276–84. doi: 10.4049/jimmunol.1202144
23. Singh A, Lelis F, Braig S, Schäfer I, Hartl D, Rieber N. Differential regulation of myeloid-derived suppressor cells by *Candida* species. *Front Microbiol*. (2016) 7:1624. doi: 10.3389/fmicb.2016.01624
24. Wu T, Zhao Y, Zhao Y. The roles of myeloid-derived suppressor cells in transplantation. *Expert Rev Clin Immunol*. (2014) 10:1385–94. doi: 10.1586/1744666X.2014.948424
25. Escors D, Liechtenstein T, Perez-Janices N, Schwarze J, Dufait I, Goyvaerts C, et al. Assessing T-cell responses in anticancer immunotherapy: dendritic cells or myeloid-derived suppressor cells? *Oncoimmunology*. (2013) 2:e26148. doi: 10.4161/onci.26148
26. Dangi A, Zhang L, Zhang X, Luo X. Murine CMV induces type 1 IFN that impairs differentiation of MDSCs critical for transplantation tolerance. *Blood Adv*. (2018) 2:669–80. doi: 10.1182/bloodadvances.2017012187
27. Paraskeva M, Bailey M, Levvey BJ, Griffiths AP, Kotsimbos TC, Williams TP, et al. Cytomegalovirus replication within the lung allograft is associated with bronchiolitis obliterans syndrome. *Am J Transplant*. (2011) 11:2190–6. doi: 10.1111/j.1600-6143.2011.03663.x
28. Yang F, Li Y, Zhang Q, Tan L, Peng L, Zhao Y. The effect of immunosuppressive drugs on MDSCs in transplantation. *J Immunol Res*. (2018) 2018:5414808. doi: 10.1155/2018/5414808
29. Wang X, Bi Y, Xue L, Liao J, Chen X, Lu Y, et al. The calcineurin-NFAT axis controls allograft immunity in myeloid-derived suppressor cells through reprogramming T cell differentiation. *Mol Cell Biol*. (2015) 35:598–609. doi: 10.1128/MCB.01251-14
30. Pallett LJ, Gill US, Quaglia A, Sinclair LV, Jover-Cobos M, Schurich A, et al. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. *Nat Med*. (2015) 21:591–600. doi: 10.1038/nm.3856

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The Emerging Role of Myeloid-Derived Suppressor Cells in Tuberculosis

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Myeloid cells are crucial for the host control of a *Mycobacterium tuberculosis* (*M.tb*) infection, however the adverse role of specific myeloid subsets has increasingly been appreciated. The relevance of such cells in therapeutic strategies and predictive/prognostic algorithms is to promote interest in regulatory myeloid cells in tuberculosis (TB). Myeloid-derived suppressor cells (MDSC) are a heterogeneous collection of phagocytes comprised of monocytic- and polymorphonuclear cells that exhibit a potent suppression of innate- and adaptive immune responses. Accumulation of MDSC under pathological conditions associated with chronic inflammation, most notably cancer, has been well-described. Evidence supporting the involvement of MDSC in TB is increasing, yet their significance in this infection continues to be viewed with skepticism, primarily due to their complex nature and the lack of genetic evidence unequivocally discriminating these cells from other terminally differentiated myeloid populations. Here we highlight recent advances in MDSC characterization and summarize findings on the TB-induced hematopoietic shift associated with MDSC expansion. Lastly, the mechanisms of MDSC-mediated disease progression and future research avenues in the context of TB therapy and prophylaxis are discussed.

Keywords: myeloid-derived suppressor cells, *Mycobacterium tuberculosis*, infectious disease, immunosuppression, innate immunity

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INTRODUCTION

Tuberculosis (TB) remains a leading cause of global mortality (1, 2). Insufficient understanding of TB disease mechanisms represents a major factor impeding its elimination (3). A recent paradigm describes TB as a continuous spectrum of processes, rather than a binary distribution between asymptomatic latent infection and active disease (3–6). This underscores the complex pathophysiology of TB, including multiple cellular effectors, regulators, and checkpoints. Myeloid cells, including neutrophils and monocytes, function both as initial effectors and during the lag phase of T-cell responses to restrict *M.tb* burden and limit disease progression by activating pro-inflammatory signaling pathways, recruiting additional phagocytes, ingesting bacilli, up-regulating bactericidal mechanisms and inducing antigen-specific adaptive immunity (7–9). Even so, myeloid cells can switch from facilitating protective immunity, to aiding pathological processes, by enhancing TB progression via immunosuppression and dysregulated inflammation (8). Chronic

mycobacterial infection triggers the generation of immunosuppressive/tolerogenic myeloid cells, which were initially referred to as "innate natural suppressor cells" (10–12). Subsequent studies have coined these as myeloid-derived suppressor cells (MDSC) (13, 14).

MYCOBACTERIA-INDUCED NATURAL SUPPRESSOR CELLS

Early reports on regulatory myeloid cells in mycobacterial infection came from *in vivo* and *in vitro* studies with *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) (11, 15–17).In these studies it was indicated that, systemic delivery of mycobacteria induce expansion of hematopoietic progenitor cells in the bone marrow, with the subsequent migration of these cells to the peritoneal cavity and their activation in the spleen (11, 18). It was further reported that BCG could induce the expansion of bone-marrow derived and splenic natural suppressor cells and that these cells could inhibit cell-mediated immunity, notably by suppressing the migratory capacity and proliferation of helper and cytotoxic T-cells (15, 16). T-cell immunosuppression was attributed to the presence of macrophage-like natural suppressor cells, the production of high levels of IL-1 and soluble suppressive factors (16, 19). Natural suppressor cells were later linked to MDSC. Natural suppressor cells from mice exposed to mycobacterial products in Complete Freud's adjuvant (CFA), shared similar phenotypic and functional features with MDSC (10). These cells highly expressed the markers of myeloid origin and differentiation, Gr-1 and CD11b, and inhibition of T-cell proliferation and IFN-γ production was linked to NO production in splenocytes (10). Subsequent studies validated the presence of MDSC during BCG infection (13) and in patients with active TB (14). Thus, initial observations of natural suppressor cells were during mycobacterial insult and established that the generation of these cells was driven by the mycobacterial products.

MDSC CHARACTERIZATION IN MYCOBACTERIAL INFECTIONS

Identification of MDSC requires a combination of assays comprising of immunophenotyping, enzyme measurements, and suppressive tests (20). Markers employed for detection of human MDSC allow, to some extent, their differentiation from monocytes and neutrophils, although this is cumbersome in mice (21). At present, three commonly reported MDSC subsets identified in human TB include early stage MDSC (e-MDSC), polymorphonuclear-MDSC (PMN-MDSC), and monocytic-MDSC (M-MDSC) (14, 22, 23). Immunosuppressive eosinophilic MDSC have recently been described during chronic *Staphylococcus aureus* infection *in vivo* but require validation in other diseases (24). MDSC enriched in TB patients, according to recent recommendations using a ficoll density-gradient (22, 23), have been classified as e-MDSC ($LIN1^{-}HLA\text{-}DR^{-/lo}CD11b^{+}CD33^{+}$), PMN-MDSC ($HLA\text{-}DR^{-/lo}CD11b^{+}CD14^{-}CD15^{+}CD33^{+/dim}$) and M-MDSC ($HLA\text{-}DR^{-/low}CD11b^{+}CD14^{+}CD15^{-}CD33^{+}$) (20). Instead of a specific subset, M-MDSC population has been described as a heterogenous population of cells, in different maturation stages (20). Since there are no specific markers for MDSC, ambiguity with other myeloid cells that have similar phenotypic characteristics and functional properties exists, especially after pathogen exposure. For instance, infection of monocytes with *Candida albicans* fungal cells and exposure to fungal components subverts monocyte differentiation to immunosuppressive dendritic cells. The phenotype of the subverted DC is characterized by the expression of CD14 with a lack of CD1a molecule, presence of CD83 and CD86 but a relatively low expression of MHC class II and CD80. These cells produce IL-12 but are associated with the release of IL-10 and IL-6 (25). Similarly our group has demonstrated that CD14+ M-MDSC production of IL-10 and IL-6 is associated with either absent, or relatively low levels of HLA-DR and CD80 (14, 26). Thus, an unequivocal marker that is able to distinguish myeloid cell population and subsets in biological samples such as whole blood culture and tissue is required. Whilst there is no specific marker for M-MDSC yet, utilization of LOX-1 as a unique PMN-MDSC marker has been proposed but (27) requires validation in TB patients.

In murine TB, PMN-MDSC are phenotypically $Gr\text{-}1{+}CD11b^{+}Ly6G^{+}Ly6C^{lo/int}$ and M-MDSC $Gr\text{-}1{+}CD11b^{+}Ly6G^{-/l0}Ly6C^{hi}$, yet functional assays are essential for their classification (28–30).

Morphological characterization has been used as a confirmatory tool to distinguish MDSC from other myeloid cells in TB samples (22, 28). Immature myeloid cells identified as PMN-MDSC share similar morphological characteristics with neutrophils, as they show ring-shaped or band nuclei. This nuclear shape can, however, be present in neutrophil progenitors and young neutrophils. Utilization of CD10 for human specimens (21) along with suppressive assays may help distinguish PMN-MDSC from non-suppressive immature neutrophils. MDSC likely encompass cells at different maturation stages with a distinct activation status and functional role. For instance, expansion of MDSC with the phenotype $Lin^{-/l0}HLA\text{-}DR^{-/lo}CD11b^{+}CD14^{+}CD33^{+}CD80^{+}$, was described in patients with active TB and their frequency correlated with disease progression (14). CD80 up-regulation upon successful TB chemotherapy was associated with MDSC differentiation into macrophages and dendritic cells (14). In mice, accumulation of an immature, heterogeneous population of $Gr1^{dim}CD11b^{+}$ cells with un-segmented nuclei, which also expresses progenitor markers ($CD117^{+}CD135^{+}$), was observed during the advanced disease in TB prone animals (28).

TISSUE COMPARTMENTALIZATION AND DYNAMICS OF MDSC IN TB

In murine models MDSC were detected in the blood during BCG vaccination (13). In adults and children suffering from TB, MDSC frequencies in the periphery were comparable to

those found in cancer patients (14). All MDSC subsets have been identified in the blood of TB patients, yet relative ratios, within different biological samples/fluids, differ in various studies (14, 22, 23). For instance, PMN-MDSC are enriched in the lung, specifically in bronchoalveolar lavage (BAL) samples of pulmonary TB patients (22) whilst the prevalence of a M-MDSC subset has been described in pleural effusions (14). Compartmentalization of the different MDSC subsets during TB in humans may be site-specific and likely dependent on the disease stage. Such an assumption is supported by findings from experimental TB. In naïve mice, MDSC can be detected at very low frequencies primarily in bone marrow. During acute TB, MDSC mildly accumulates in the lung and upon disease progression their numbers dramatically increase in all aforementioned organs and are also detected in the blood (28). High levels of MDSC in bone-marrow suggests that their genesis occurs primarily via medullary hematopoiesis. A pro-inflammatory environment, abundant in IL-6/G-CSF/PROK-2 may promote myelo- and granulopoiesis, whereas recruitment of MDSC to the lung could be directed by abundant S100-proteins/MMP-9/G-CSF (20, 29). Accumulation of MDSC in the lung parenchyma parallels TB progression in susceptible mice (29, 30). In *M.tb*-infected-necrosis prone mice, M-MDSC accumulate at the edges of necrotic granulomas (30). A recent study further strengthened the case for MDSC as regulators of granuloma biology. Human *ex vivo* generated M-MDSC promote mycobacterial replication in *in vitro* established granulomas, in a process dependent on abundant release of IL-10 (26).

Dynamics of MDSC subsets through-out the course of the TB disease spectrum (31) are relevant for disease pathophysiology. In TB patients, MDSC abundances have not yet been clearly linked with the extent of disease, e.g., by establishing a correlation between their frequencies and lung radiological involvement, smear grading or bacterial burden. Community controls from a high-exposure region and also individuals with remote exposure to *M.tb*, display very low levels of circulating MDSC, yet frequencies of MDSC increase in recently exposed house hold contacts (HHC) of TB patients (19). MDSC presumably emerge in incipient TB with their increased frequency associated with disease progression. TB-resistant mice that are devoid of necrotic granulomas have minimal levels of MDSC, whilst necrotic prone mouse strains NOS2$^{-/-}$ (knock-out), C3HeB/FeJ, 129S2 (immunocompetent) exhibit higher frequencies with the highest levels observed in immunodeficient (RAG2$^{-/-}$) animals (29, 30). The accumulation of MDSC in necrotic granulomas has been associated with the inability to control *M.tb* infection and lung pathology (28, 29). Pulmonary tuberculosis manifests differently than pleural tuberculosis and MDSC biology in pleural cavities still needs further characterization. In TB patients, MDSC are present in pleural effusions and blood and the immunosuppressive potential of MDSC from individuals with a long term infection exceeds the suppression of cells isolated from people with recent *M.tb* exposure, which also affects CD8 T-cell responsiveness (14). Upon a successful cure, MDSC frequencies decrease to levels observed in healthy controls (14). In children, completion of standard TB treatment was not accompanied by a MDSC decline, likely reflecting the more complex disease presentation of pediatric TB and possibly the polarization of the immune response which may be different to adult immune response (32).

MDSC DIRECTLY INTERACT WITH MYCOBACTERIA

Lung-residing M-MDSC harbor *M.tb* and promote bacterial growth through mechanisms involving IL-4/IL4Rα signaling (29). Despite the production of nitric oxide (NO), a potent anti-mycobacterial molecule, MDSC are inefficient at controlling mycobacterial growth (13). Although *ex vivo* generated human MDSC are not able to provide a niche for fast replication of *M.tb* when compared to macrophages, they do however exert a potent suppressive activity against T-cells upon infection (26).Recent reports indicate that myeloid cell ontogeny affects their capacity to support mycobacterial growth. Interstitial macrophages, supposedly originating from circulating monocytes, allow lower *M.tb*. replication rates as compared to fetal germline derived alveolar macrophages (AM). This phenomenon has been linked to the dramatically different metabolic states of AM and interstitial macrophages, with highly up-regulated fatty acid uptake and β-oxidation vs. high glycolytic activity, respectively (33). Pre-existing metabolic bias of myeloid cells controls *M.tb* growth (33). Of note, tumor-infiltrating MDSC preferentially use fatty acid-β-oxidation (FAO) as a primary energy source, display up-regulation in FAO genes and increases the oxygen consumption rate (34). We, and others have previously shown that MDSC are capable of mycobacterial internalization, however, they display poor microbicidal activity (13, 26). Considering that *M.tb* uses host fatty acids and cholesterol, the metabolic status of MDSC likely offers a nutritional niche supporting *M.tb* maintenance (35, 36). Whether FAO affects *M.tb* survival within MDSC remains to be validated. In the same vein, the metabolic state of *M.tb* as well as its subcellular localization within MDSC are largely unknown and should be defined.

MEDIATORS OF MDSC EXPANSION AND ACTIVATION IN TB

Expansion and activation of MDSC is mediated by chronic, low-grade inflammation, resulting in the pathological activation of myeloid cells (37). Currently, it is difficult to discriminate signals mediating MDSC expansion from those mediating MDSC activation. Recent findings support a two-step process involving cellular expansion, licensing, and activation (37, 38). First, chronic exposure to GM-CSF, IL-6, prostaglandins, and alarmins such as S100A8/9 (38, 39) promote "emergency myelopoiesis," impede on terminal maturation of myeloid progenitors. The second phase involves activation of these "licensed" myeloid cells, through the panoply of inflammatory cytokines (e.g., IFN-γ, IL-1β, IL- 6, TNF-α, IL-4), DAMPs (e.g., HMGB1), and likely also PAMPs (e.g., LPS) to obtain suppressive functions (37–39). Such factors are produced during TB and enriched in TB-susceptible mice accumulating MDSC (**Figure 1A**) (29). Additional molecules detected in TB lesions,

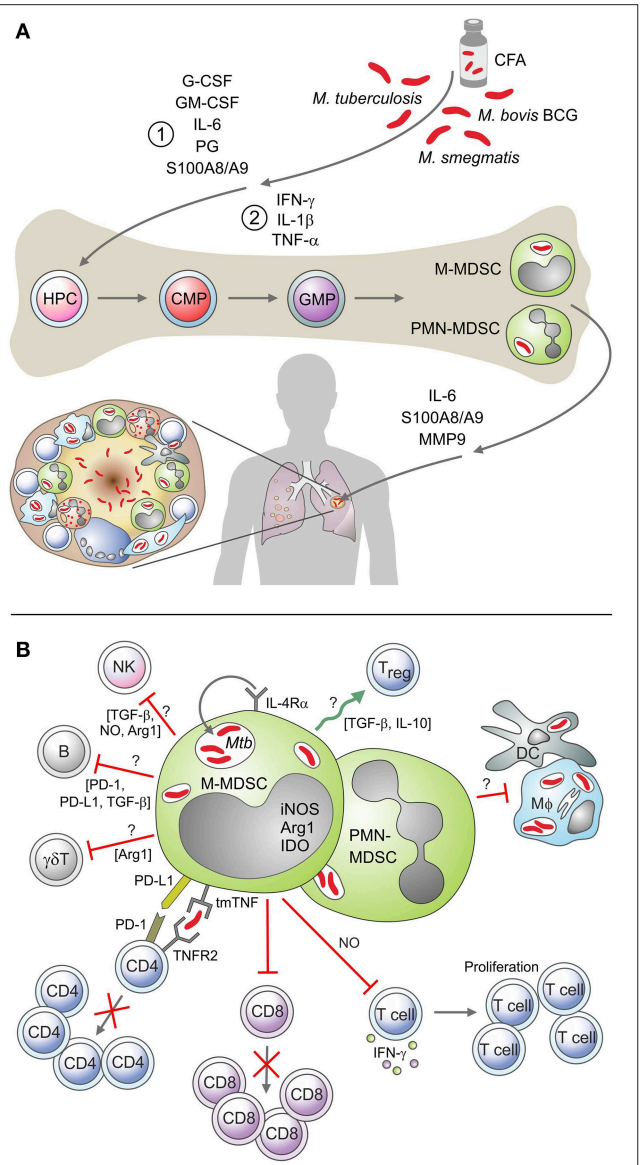

FIGURE 1 | (A) Mediators of MDSC expansion and activation in a mycobacterial environment. A schematic depicting mediators associated with the proposed "two-signal" MDSC expansion and activation process, in a mycobacterial environment. These include cytokines, chemokines, calcium-binding proteins, and matrix metalloproteinases. **(B)** MDSC cellular interaction and mediators of immunosuppression in a mycobacterial environment. Examples of the known and suggested interactions of MDSC with other immune cells in the mycobacterial setting, including the soluble mediators associated with their immunosuppressive activity.

including prokineticin 2 (PROK 2) and MMP9, which promote MDSC accumulation in target organs, may also regulate MDSC expansion (29). Recent reports indicate that transmembrane TNF-alpha regulates the activation and expansion of PMN-MDSC and M-MDSC in the pleural cavity of BCG infected mice (40). In mycobacterial infections, M-MDSC are induced regardless of key virulence factors, as *M.tb, M.smeg,* and BCG have proven to induce MDSC (13). Consequently, due their immunosuppressive activity and high frequency during disease progression, MDSC have been identified as one of the factors that may contribute to a low BCG vaccine efficacy (41). Other factors may include geographical location, helminthic co-infection, route of BCG administration and mycobacterial strain (42). It is important to note that the robust cytokine response often observed following BCG vaccination, contradicts the MDSC functions described above. We suspect that this perceived discrepancy, could be ascribed to the requirement of a 2nd activation signal or the mycobacterial strain-specific differences on MDSC function. Alternatively, the MDSC suppressive function might stretch beyond T-cell immunity and affect other cell subsets which are rarely evaluated following BCG vaccination, with the route of the vaccination and the age of the vaccine, also contributing to the outcome. The role of live bacteria in regions from which MDSC originate, such as immature bone marrow cells, still need to be investigated.

Mycobacterial glycolipids contained in CFA promote the expansion of the MDSC (10). A comprehensive comparison of "licensed" monocytes, M-MDSC and additional monocytic subsets present in the *M.tb* infected lung is necessary to distinguish pathways driving MDSC genesis. Advanced techniques such as quantitative shotgun proteomics, RNASeq and chromatin ATAC mapping should provide insights into potentially discriminating markers and differentiation pathways.

MDSC IMMUNOSUPPRESSIVE MECHANISMS DURING TB INFECTION

MDSC exert their immunosuppressive activity through mechanisms that involve soluble factors, cell membrane molecules and the modulation of local concentrations off of metabolites and amino acid (20, 43). Most studies focus on T-cell suppression (ref), however MDSC also interact with macrophages and dendritic cells, and induce regulatory B- and T-cells (44–46). Such interactions have not yet been considered in TB (**Figure 1B**). The interaction of MDSC with T-cells has been established in TB patients, though the effects on antigen-specific responder lymphocytes still await clarification. Suppression of polyclonal stimulated CD4 and CD8 T-cells involves the inhibition of cytokine production, T-cell activation and modulation of T-cell trafficking (14). Whereas, PMN-MDSC expansion correlates with abundant plasma NO (22), phenotypically resembling MDSC present abundant indoleamine 2,3-dioxygenase (IDO) and arginase-1 (ARG-1) (32). In BCG vaccinated mice, iNOS-mediated tendency of MDSC to dampen T-cell priming, suppress polyclonal T-cell proliferation and IFN-γ release (13). iNOS mediates the suppression of lymphocytes also in murine TB, though *in situ* co-expression of ARG1 and iNOS has been detected in lung lesions (29).Cell surface molecules involved in the regulation of MDSC functions have been identified in experimental TB studies. In mice with mycobacterial pleurisy, tmTNF-α regulates MDSC activity through the cell-to-cell interaction between tmTNF-α expressing MDSC and TNFR2 expressing CD4 T-cells (40). Human MDSC up-regulate PD-L1 upon *in vitro* mycobacterial infection (26) and

employ this check-point molecule to restrict T-cell proliferation (26, 47). IFN-γ counteracts PD-L1 induced suppression (47) and this may explain the profound immunosuppression in end stage TB patients. Relevance of additional enzymes enriched in MDSC purified from cancer patients, such as NADPH and COX2 (20), as well as roles of autophagy molecules (48), remain to be established in TB. Of paramount importance will be the deciphering of interactions between MDSC and macrophages, as those cells harbor and aid restricting bacillary replication. The capacity of MDSC to modulate Treg dynamics, induce Breg and alter NK activity in TB is also unknown. High dimensional analyses, e.g., mass cytometry and histo-cytometry could establish effects on MDSC on various immune cells and facilitate the in-depth functional characterization of these cells. MDSC may further contribute to TB reactivation by exacerbating the immunosuppressive effects of immunotherapy such as anti-TNF agents, absence of TNF-alpha has been associated with an increased bacterial load and T-cell immunosuppression (49, 50).

MDSC AND TB CO-MORBIDITIES

Diseases promoting TB development are typically linked to immunosuppression or dysregulation of immunity and encompass HIV (51, 52) and diabetes (53, 54). In addition, undernourishment, alcoholism, and smoking are considered risk factors for TB. Currently, the precise role of MDSC in these conditions and subsequent implications for TB are not clear. MDSC have been reported in HIV infection, but a prevalence of distinct subsets during co-infection has not been unanimously established. Some studies report high frequencies of the PMN-MDSC subset (52, 55–57) whilst others describe increased M-MDSC populations in AIDS patients (58–61). MDSC frequencies correlate with AIDS progression and viral load (51, 59), while anti-retroviral therapy (ART) reduces systemic MDSC frequencies (44, 62, 63). Even HIV exposed uninfected children display abundant circulating MDSC (32). MDSC activity in an HIV environment involves enhanced IL-10 production, induction of CD4+CD25+FoxP3+Tregs and suppression of T-cell responses, notably inhibition of IFN-gamma release by autologous T-cells (52, 60). Such effects may contribute to development of TB in LTBI people infected with HIV, however further studies are required to elucidate the precise role of HIV-induced MDSC in TB reactivation. Very few reports focus on MDSC in diabetes. Recent trials suggest a beneficial effect with MDSC protecting against the development of type-2 diabetes (T2DM) in humans (64). Interestingly, the anti-diabetic drug metformin, showing efficacy as an adjunct therapy in TB (65), causes reduction of MDSC in cancer patients (66). Metformin's effect on MDSC in TB patients has not been evaluated. Smoking is regarded as a predisposing factor that can accelerate TB progression. Although smoking has been associated with MDSC expansion and generation in COPD patients (67, 68), the role of these cells in TB is not clear and should be clarified. Obesity-driven chronic, low-grade inflammation and leptin interaction has also shown to induce MDSC that, although protective against some metabolic dysfunctions, appear to be detrimental to tumor progression (69). At the other end of the spectrum, malnutrition has also been correlated to MDSC induction, suggesting a link with diseases characterized by wasting and malnutrition, such as TB (70). It is tempting to speculate that enhanced MDSC levels in diseases and conditions causing alterations in immune reactivity may contribute to TB reactivation, however this remains to be tested.

THERAPEUTIC STRATEGIES TARGETING MDSC IN TB

Shortly after identification of MDSC in TB patients and murine models, these cells emerged as promising targets for adjunct host-directed therapy (HDT) approaches (8, 41, 71). The focus of such strategies has been to reverse the impact of MDSC on T-cell immunity in TB by implementing host modulating therapeutic strategies such as those blocking MDSC induction or activation, inhibiting MDSC function or reversing their suppressive function. These strategies have been recently reviewed elsewhere (71). More recently, denileukin diftitox, an anti-neoplastic agent comprised of IL-2 and Diphtheria toxin, potentiates standard TB treatment in a mouse model through the elimination of MDSC and Treg (72). Similarly, combined immunotherapy consisting of ATRA and alpha galactosylceramide as an adjunct immunotherapy improved standard TB treatment (73). Other studies on ATRA have reported the reduction of MDSC and increase in T-cell number with an impact on bacillary loads and lung pathology (13, 29). Tasquinimod (TSQ), a quinoline-3-carboxyamide analog, targets S100A9, a molecule which has been implicated in MDSC accumulation and function. TSQ is in clinical development for the treatment of various cancers and has recently shown to significantly enhance the antitumor effects of immunotherapeutics in cancer mouse models, by inhibiting the suppressive function of MDSC and tumor-associated macrophages (TAM) (74). More recently, TSQ treatment in an acute mouse model of TB, enhanced *M.tb* clearance, reduced Treg and MDSC frequencies and enhanced the efficacy of the standard treatment regimen (75).

Cytokines indirectly affect MDSC accumulation/function and a recent study has shown that IFN-γ decreases the suppressive function of MDSC by reducing the arginase activity suppressing PD-1/PD-L1 (47). Although not yet tested in TB, a combination treatment of IL-17R and IFN-γ has shown potential in cancer, by reducing the levels of MDSC and increasing T-cells (76). Other MDSC targeting agents tested in cancer, which have shown potential in TB, but with unknown effects on MDSC, include metformin, tyrosine kinase inhibitors (imatinib), PDE-5 inhibitors, and arginase inhibitors (71). The COX-2 inhibitor, etoricoxib, is currently evaluated as HDT for TB and its effect on MDSC levels will be considered in the trial (NCT02503839).

CONCLUSION

The MDSC arena has experienced several research advances in the context of infectious diseases. Nonetheless, the complex and protracted nature of *M.tb* infection along with challenges in biology of MDSC research have delayed comprehensive investigations on MDSC in the TB field. Ultimately, MDSC research in TB would be insignificant without an eventual

tangible contribution to the clinical benefit of patients. Development of immunotherapies targeting MDSC is undergoing a slow but steady progress, however many TB HDT trials fail to consider the impact of these treatments on MDSC function and frequency. The lack of compounds targeting MDSC specifically, contributes to this problem. The safety, efficacy, dose, and timing of interventions targeting MDSC in TB, will also require careful evaluation, and so too will the effect of novel neonatal vaccines and adult re-vaccination strategies on MDSC genesis. Greater focus on these and other MDSC knowledge gaps is expected to accelerate the discovery of effective TB immunotherapies, thereby contributing to an increased TB cure rate, more durable clinical responses and superior control of drug-resistant *M.tb* strains.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Raviglione M, Sulis G. Tuberculosis 2015: burden, challenges and strategy for control and elimination. *Infect Dis Rep*. (2016) 8:6570. doi: 10.4081/idr.2016.6570
2. World Health Organization. *Global Tuberculosis Report 2018*. World Health Organization (2018).
3. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. *Clin Microbiol Rev*. (2018) 31:e00021–18. doi: 10.1128/CMR.00021-18
4. Barry CE, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol*. (2009) 7:845–55. doi: 10.1038/nrmicro2236
5. Delogu G, Goletti D. The spectrum of tuberculosis infection: new perspectives in the era of biologics. *J Rheumatol Suppl*. (2014) 91:11–16. doi: 10.3899/jrheum.140097
6. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. *Nat Rev Dis Primers*. (2016) 2:16076. doi: 10.1038/nrdp.2016.76
7. Srivastava S, Ernst JD, Desvignes L. Beyond macrophages: the diversity of mononuclear cells in tuberculosis. *Immunol Rev*. (2014) 262:179–92. doi: 10.1111/imr.12217
8. Dorhoi A, Kaufmann SHE. Versatile myeloid cell subsets contribute to tuberculosis-associated inflammation: HIGHLIGHTS. *Eur J Immunol*. (2015) 45:2191–202. doi: 10.1002/eji.201545493
9. Liu Y, Wang R, Jiang J, Cao Z, Zhai F, Sun W, et al. A subset of CD1c+ dendritic cells is increased in patients with tuberculosis and promotes Th17 cell polarization. *Tuberculosis*. (2018) 113:189–99. doi: 10.1016/j.tube.2018.10.007
10. Wang Z, Wang H, Jiang J, Li Z, Zhang J, Qin Z. A Myeloid cell population induced by freund adjuvant suppresses T-cell mediated antitumor immunity. *J Immunother*. (2010) 33:167–77. doi: 10.1097/CJI.0b013e3181bed2ba
11. Bennett JA, Rao VS, Mitchell MS. Systemic Bacillus Calmette-Guerin (BCG) activates natural suppressor cells. *Proc Natl Acad Sci USA*. (1978) 75:5142–4. doi: 10.1073/pnas.75.10.5142
12. Kato K, Yamamoto K-I. Suppression of BCG cell wall-induced delayed-type hypersensitivity by BCG pre-treatment. II. Induction of suppressor T cells by heat-killed BCG injection. *Immunology*. 45:655–61.
13. Martino A, Badell E, Abadie V, Balloy V, Chignard M, Mistou M-Y, et al. *Mycobacterium bovis* bacillus calmette-guérin vaccination mobilizes innate myeloid-derived suppressor cells restraining *in vivo* T cell priming via IL-1R-dependent nitric oxide production. *J Immunol*. (2010) 184:2038–47. doi: 10.4049/jimmunol.0903348
14. du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, et al. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after recent *Mycobacterium tuberculosis* infection suppresses T-cell function. *Am J Respir Crit Care Med*. (2013) 188:724–32. doi: 10.1164/rccm.201302-0249OC
15. Kato K, Yamamoto K. Suppression of BCG cell wall-induced delayed-type hypersensitivity by BCG pre-treatment. II. Induction of suppressor T cells by heat-killed BCG injection. *Immunology*. (1982) 45:655–61.
16. Klimpel G, Okada M, Henney C. Inhibition of *in vitro* cytotoxic responses by BCG-induced macrophage-like suppressor cells. II. Suppression occurs at the level of a "helper" T cell. *J Immunol*. (1979) 123:350–7.
17. Mellow L, Sabbadini E. Effect of Bacillus Calmette-Guerin on the *in vitro* generation of cytotoxic T lymphocytes. *Immunology*. (1985) 56:235–43.
18. Bennett J, Marsh J. Relationship of Bacillus Calmette-Guérin-induced suppressor cells to hematopoietic precursor cells. *Cancer Res*. (1980) 40:80–5.
19. Kendall L, Sabbadini E. Effect of Bacillus Calmette-Guérin on the *in vitro* generation of cytotoxic T lymphocytes. I. Effect of BCG on the frequency of cytotoxic T lymphocyte precursors and on the production of helper factors. *J Immunol*. (1981) 127:234–8.
20. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun*. (2016) 7:12150. doi: 10.1038/ncomms12150
21. Cassetta L, Baekkevold E, Brandau S, Bujiko A, Cassatella M, Dorhoi A, et al. Deciphering myeloid-derived suppressor cells: isolation and markers in humans, mice and non-human primates. *Cancer Immunol Immunother*. (2019) 68:687–97. doi: 10.1007/s00262-019-02302-2
22. El Daker S, Sacchi A, Tempestilli M, Carducci C, Goletti D, Vanini V, et al. Granulocytic myeloid derived suppressor cells expansion during active pulmonary tuberculosis is associated with high nitric oxide plasma level. *PLoS ONE*. (2015) 10:e0123772. doi: 10.1371/journal.pone.0123772
23. Yang B, Wang X, Jiang J, Zhai F, Cheng X. Identification of CD244-expressing myeloid-derived suppressor cells in patients with active tuberculosis. *Immunol Lett*. (2014) 158:66–72. doi: 10.1016/j.imlet.2013.12.003
24. Goldmann O, Beineke A, Medina E. Identification of a novel subset of myeloid-derived suppressor cells during chronic staphylococcal infection that resembles immature eosinophils. *J Infect Dis*. (2017) 216:1444–51. doi: 10.1093/infdis/jix494

25. Nisini R, Torosantucci A, Romagnoli G, Chiani P, Donati S, Gagliardi M, et al. Glucan of *Candida albicans* cell wall causes the subversion of human monocyte differentiation into dendritic cells. *J Leukoc Biol*. (2007) 82:1136–42. doi: 10.1189/jlb.0307160
26. Agrawal N, Streata I, Pei G, Weiner J, Kotze L, Bandermann S, et al. Human monocytic suppressive cells promote replication of *Mycobacterium tuberculosis* and alter stability of *in vitro* generated granulomas. *Front Immunol*. (2018) 9:2417. doi: 10.3389/fimmu.2018.02417
27. Condamine T, Dominguez GA, Youn J-I, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol*. (2016) 1:aaf8943. doi: 10.1126/sciimmunol.aaf8943
28. Tsiganov EN, Verbina EM, Radaeva TV, Sosunov VV, Kosmiadi GA, Nikitina IY, et al. Gr-1dimCD11b+ immature myeloid-derived suppressor cells but not neutrophils are markers of lethal tuberculosis infection in mice. *J Immunol*. (2014) 192:4718–27. doi: 10.4049/jimmunol.1301365
29. Knaul JK, Jörg S, Oberbeck-Mueller D, Heinemann E, Scheuermann L, Brinkmann V, et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. *Am J Respir Crit Care Med*. (2014) 190:1053–66. doi: 10.1164/rccm.201405-0828OC
30. Obregón-Henao A, Henao-Tamayo M, Orme IM, Ordway DJ. Gr1intCD11b+ Myeloid-derived suppressor cells in *Mycobacterium tuberculosis* infection. *PLoS ONE*. (2013) 8:e80669. doi: 10.1371/journal.pone.0080669
31. Cadena AM, Fortune SM, Flynn JL. Heterogeneity in tuberculosis. *Nat Rev Immunol*. (2017) 17:691–702. doi: 10.1038/nri.2017.69
32. Du Plessis N, Jacobs R, Gutschmidt A, Fang Z, van Helden PD, Lutz MB, et al. Phenotypically resembling myeloid derived suppressor cells are increased in children with HIV and exposed/infected with *Mycobacterium tuberculosis*: immunity to infection. *Eur J Immunol*. (2017) 47:107–18. doi: 10.1002/eji.201646658
33. Huang L, Nazarova EV, Tan S, Liu Y, Russell DG. Growth of *Mycobacterium tuberculosis in vivo* segregates with host macrophage metabolism and ontogeny. *J Exp Med*. (2018) 215:1135–52. doi: 10.1084/jem.20172020
34. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *Cancer Immunol Res*. (2015) 3:1236–47. doi: 10.1158/2326-6066.CIR-15-0036
35. Daniel J, Maamar H, Deb C, Sirakova T, Kolattukudy P. *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. *PLoS Pathog*. (2011) 7:e1002093. doi: 10.1371/journal.ppat.1002093
36. Guirado E, Mbawuike U, Keiser TL, Arcos J, Azad AK, Wang S-H, et al. Characterization of host and microbial determinants in individuals with latent tuberculosis infection using a human granuloma model. *mBio*. (2015) 6:e02537–14. doi: 10.1128/mBio.02537-14
37. Ribechini E, Hutchinson JA, Hergovits S, Heuer M, Lucas J, Schleicher U, et al. Novel GM-CSF signals via IFN-γR/IRF-1 and AKT/mTOR license monocytes for suppressor function. *Blood Adv*. (2017) 1:947–60. doi: 10.1182/bloodadvances.2017006858
38. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res*. (2017) 5:3–8. doi: 10.1158/2326-6066.CIR-16-0297
39. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol*. (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
40. Chavez-Galan L, Vesin D, Uysal H, Blaser G, Benkhoucha M, Ryffel B, et al. Transmembrane tumor necrosis factor controls myeloid-derived suppressor cell activity via TNF receptor 2 and protects from excessive inflammation during BCG-induced pleurisy. *Front Immunol*. (2017) 8:999. doi: 10.3389/fimmu.2017.00999
41. Jayashankar L, Hafner R. Adjunct strategies for tuberculosis vaccines: modulating key immune cell regulatory mechanisms to potentiate vaccination. *Front Immunol*. (2016) 7:577. doi: 10.3389/fimmu.2016.00577
42. Moliva J, Turner J, Torrelles J. Immune responses to Bacillus-Calmette-Guerin vaccination: why do they fail to protect against *Mycobacterium tuberculosis*? *Front Immunol*. (2017) 8:407. doi: 10.3389/fimmu.2017.00407
43. Dorhoi A, du Plessis N. Monocytic myeloid-derived suppressor cells in chronic infections. *Front Immunol*. (2018) 8:1895. doi: 10.3389/fimmu.2017.01895
44. Lei G-S, Zhang C, Lee C-H. Myeloid-derived suppressor cells impair alveolar macrophages through PD-1 receptor ligation during Pneumocystis pneumonia. *Infect Immun*. (2015) 83:572–82. doi: 10.1128/IAI.02686-14
45. Park M-J, Lee S-H, Kim E-K, Lee E-J, Park S-H, Kwok S-K, et al. Myeloid-derived suppressor cells induce the expansion of regulatory B cells and ameliorate autoimmunity in the Sanroque mouse model of systemic lupus erythematosus. *Arthritis Rheumatol*. (2016) 68:2717–27. doi: 10.1002/art.39767
46. Wang Y, Schafer CC, Hough KP, Tousif S, Duncan SR, Kearney JF, et al. Myeloid-derived suppressor cells impair B cell responses in lung cancer through IL-7 and STAT5. *J Immunol*. (2018) 201:278–95. doi: 10.4049/jimmunol.1701069
47. Zhan X, Fang Y, Hu S, Wu Y, Yang K, Liao C, et al. IFN-γ differentially regulates subsets of Gr-1+CD11b+ myeloid cells in chronic inflammation. *Mol Immunol*. (2015) 66:451–62. doi: 10.1016/j.molimm.2015.05.011
48. Alissafi T, Hatzioannou A, Mintzas K, Barouni RM, Banos A, Sormendi S, et al. Autophagy orchestrates the regulatory program of tumor-associated myeloid-derived suppressor cells. *J Clin Invest*. (2018) 128:3840–52. doi: 10.1172/JCI120888
49. Clay H, Volkman H, Ramakrishnan L. TNF signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death but not tuberculous granuloma formation. *Immunity*. (2008) 29:283–94. doi: 10.1016/j.immuni.2008.06.011
50. Miller A, Ernst J. Anti-TNF immunotherapy and tuberculosis reactivation: another mechanism revealed. *J Clin Invest*. (2009) 119:1079–82. doi: 10.1172/JCI39143
51. Vollbrecht T, Stirner R, Tufman A, Roider J, Huber RM, Bogner JR, et al. Chronic progressive HIV-1 infection is associated with elevated levels of myeloid-derived suppressor cells. *AIDS*. (2012) 26:F31–7. doi: 10.1097/QAD.0b013e328354b43f
52. Tumino N, Turchi F, Meschi S, Lalle E, Bordoni V, Casetti R, et al. In HIV-positive patients, myeloid-derived suppressor cells induce T-cell anergy by suppressing CD3ζ expression through ELF-1 inhibition. *AIDS*. (2015) 29:2397–407. doi: 10.1097/QAD.0000000000000871
53. Faurholt-Jepsen D, Range N, PrayGod G, Jeremiah K, Faurholt-Jepsen M, Aabye M, et al. Diabetes is a strong predictor of mortality during tuberculosis treatment: a prospective cohort study among tuberculosis patients from Mwanza, Tanzania. *Trop Med Int Health*. (2013) 18:822–9. doi: 10.1111/tmi.12120
54. Gil-Santana L, Almeida-Junior J, Oliveira C, Hickson L, Daltro C, Castro S, et al. Diabetes is associated with worse clinical presentation in tuberculosis patients from Brazil: a retrospective cohort study. *PLoS ONE*. (2016) 11:e0146876. doi: 10.1371/journal.pone.0146876
55. Zhang Z-N, Yi N, Zhang T-W, Zhang L-L, Wu X, Liu M, et al. Myeloid-derived suppressor cells associated with disease progression in primary HIV infection: PD-L1 blockade attenuates inhibition. *J Acquir Immune Defic Syndr*. (2017) 76:200–8. doi: 10.1097/QAI.0000000000001471
56. Tumino N, Bilotta MT, Pinnetti C, Ammassari A, Antinori A, Turchi F, et al. Granulocytic myeloid-derived suppressor cells increased in early phases of primary HIV infection depending on TRAIL plasma level. *J Acquir Immune Defic Syndr*. (2017) 74:575–82. doi: 10.1097/QAI.0000000000001283
57. Bowers NL, Helton ES, Huijbregts RPH, Goepfert PA, Heath SL, Hel Z. Immune Suppression by neutrophils in HIV-1 infection: role of PD-L1/PD-1 pathway. *PLoS Pathog*. (2014) 10:e1003993. doi: 10.1371/journal.ppat.1003993
58. Gama L, Shirk E, Russell J, Carvalho K, Li M, Queen S, et al. Expansion of a subset of CD14highCD16negCCR2low/neg monocytes functionally similar to myeloid-derived suppressor cells during SIV and HIV infection. *J Leukoc Biol*. (2012) 91:803–16. doi: 10.1189/jlb.1111579
59. Qin A, Cai W, Pan T, Wu K, Yang Q, Wang N, et al. Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals. *J Virol*. (2013) 87:1477–90. doi: 10.1128/JVI.01759-12
60. Garg A, Spector SA. HIV type 1 gp120-induced expansion of myeloid derived suppressor cells is dependent on interleukin 6 and suppresses immunity. *J Infect Dis*. (2014) 209:441–51. doi: 10.1093/infdis/jit469
61. Wang L, Zhao J, Ren JP, Wu XY, Morrison ZD, El Gazzar M, et al. Expansion of myeloid-derived suppressor cells promotes differentiation

of regulatory T cells in HIV-1+ individuals. *AIDS*. (2016) 30:1521–31. doi: 10.1097/QAD.0000000000001083
62. Meng Z, Du L, Hu N, Byrd D, Amet T, Desai M, et al. Antiretroviral therapy normalizes autoantibody profile of HIV patients by decreasing CD33+CD11b+HLA-DR+ cells: a cross-sectional study. *Medicine*. (2016) 95:e3285. doi: 10.1097/MD.0000000000003285
63. Grützner EM, Hoffmann T, Wolf E, Gersbacher E, Neizert A, Stirner R, et al. Treatment intensification in HIV-infected patients is associated with reduced frequencies of regulatory T cells. *Front Immunol*. (2018) 9:811. doi: 10.3389/fimmu.2018.00811
64. Wang T, Wen Y, Fan X. Myeloid-derived suppressor cells suppress CD4+ T cell activity and prevent the development of type 2 diabetes. *Acta Biochim Biophys Sin*. (2018) 50:362–9. doi: 10.1093/abbs/gmy014
65. Restrepo BI. Metformin: candidate host-directed therapy for tuberculosis in diabetes and non-diabetes patients. *Tuberculosis*. (2016) 101:S69–72. doi: 10.1016/j.tube.2016.09.008
66. Li L, Wang L, Li J, Fan Z, Yang L, Zhang Z, et al. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res*. (2018) 78:1779–91. doi: 10.1158/0008-5472.CAN-17-2460
67. Scrimini S, Pons J, Agustí A, Clemente A, Sallán M, Bauçà J, et al. Expansion of myeloid-derived suppressor cells in chronic obstructive pulmonary disease and lung cancer: potential link between inflammation and cancer. *Cancer Immunol Immunother*. (2015) 64:1261–70. doi: 10.1007/s00262-015-1737-x
68. Scrimini S, Pons J, Agustí A, Soriano JB, Cosio BG, Torrecilla JA, et al. Differential effects of smoking and COPD upon circulating myeloid derived suppressor cells. *Respir Med*. (2013) 107:1895–903. doi: 10.1016/j.rmed.2013.08.002
69. Clements VK, Long T, Long R, Figley C, Smith DMC, Ostrand-Rosenberg S. Frontline science: high fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. *J Leukoc Biol*. (2018) 103:395–407. doi: 10.1002/JLB.4HI0517-210R
70. Gonda K, Shibata M, Ohtake T, Matsumoto Y, Tachibana K, Abe N, et al. Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. *Oncol Lett*. (2017) 14:1766–74. doi: 10.3892/ol.2017.6305
71. du Plessis N, Kotze LA, Leukes V, Walzl G. Translational potential of therapeutics targeting regulatory myeloid cells in tuberculosis. *Front Cell Infect Microbiol*. (2018) 8:332. doi: 10.3389/fcimb.2018.00332
72. Gupta S, Cheung L, Pokkali S, Winglee K, Guo H, Murphy J, et al. Suppressor cell-depleting immunotherapy with Denileukin Diftitox is an effective host-directed therapy for tuberculosis. *J Infect Dis*. (2017) 215:1883–7. doi: 10.1093/infdis/jix208
73. Mourik B, Leenen P, de Knegt G, Huizinga R, van der Eerden B, Wang J, et al. Immunotherapy added to antibiotic treatment reduces relapse of disease in a mouse model of tuberculosis. *Am J Respir Cell Mol Biol*. (2017) 56:233–41. doi: 10.1165/rcmb.2016-0185OC
74. Shen L, Sundstedt A, Ciesielski M, Miles KM, Celander M, Adelaiye R, et al. Tasquinimod modulates suppressive myeloid cells and enhances cancer immunotherapies in murine models. *Cancer Immunol Res*. (2015) 3:136–48. doi: 10.1158/2326-6066.CIR-14-0036
75. Gupta S, Krug S, Pokkali S, Leanderson T, Isaacs JT, Srikrishna G, et al. Pharmacologic exhaustion of suppressor cells with tasquinimod enhances bacterial clearance during tuberculosis. *Am J Respir Crit Care Med*. (2018) 199:386–89. doi: 10.1164/rccm.201805-0820LE
76. He D, Li H, Yusuf N, Elmets CA, Li J, Mountz JD, et al. IL-17 promotes tumor development through the induction of tumor promoting microenvironments at tumor sites and myeloid-derived suppressor cells. *J Immunol*. (2010) 184:2281–8. doi: 10.4049/jimmunol.0902574

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REVIEW
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Myeloid-Derived Suppressor Cells: Ductile Targets in Disease

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Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of immature myeloid cells with major regulatory functions and rise during pathological conditions, including cancer, infections and autoimmune conditions. MDSC expansion is generally linked to inflammatory processes that emerge in response to stable immunological stress, which alter both magnitude and quality of the myelopoietic output. Inability to reinstate physiological myelopoiesis would fall in an "emergency state" that perpetually reprograms myeloid cells toward suppressive functions. While differentiation and reprogramming of myeloid cells toward an immunosuppressive phenotype can be considered the result of a multistep process that originates in the bone marrow and culminates in the tumor microenvironment, the identification of its driving events may offer potential therapeutic approaches in different pathologies. Indeed, whereas expansion of MDSCs, in both murine and human tumor bearers, results in reduced immune surveillance and antitumor cytotoxicity, placing an obstacle to the effectiveness of anticancer therapies, adoptive transfer of MDSCs has shown therapeutic benefits in autoimmune disorders. Here, we describe relevant mechanisms of myeloid cell reprogramming leading to generation of suppressive MDSCs and discuss their therapeutic ductility in disease.

Keywords: emergency myelopoiesis, myeloid-derived suppressor cells (MDSCs), immunosuppression, cancer, autoimmune diseases

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INTRODUCTION

Immunologic stress, such as infection and cancer, modifies the magnitude and composition of the hematopoietic output, a feature of immune regulation defined as "emergency" hematopoiesis, to guarantee proper supply of both lymphoid and myeloid cells to increased demand (1). Under steady-state conditions, myelopoiesis is a strictly regulated process that consists of a series of cell lineage commitments, encompassing sequential steps of differentiation that govern the transition of hematopoietic stem cells (HSCs) to myeloid precursors and then to mature immune cells, which is necessary to maintain the physiological levels of circulating neutrophils and monocytes (2). This highly coordinated process is orchestrated by cytokines and growth factors, which act through activation of specific transcription factors that differentially drive terminal differentiation of myeloid cells. In particular, whereas C/EBPα appears to be a major regulator of "steady-state" granulopoiesis (3), C/EBPβ (4) and Signal Transducer and Activator of Transcription 3 (STAT3) (5) promote expansion and maturation of neutrophils in emergency conditions. Moreover,

interleukin-17A (IL-17A) promotes both granulocyte-colony stimulating factor (G-CSF)- and stem-cell-factor-mediated neutrophilia (6) and supports G-CSF-driven "emergency" myelopoiesis (7). Terminal macrophage differentiation is instead induced by macrophage-CSF (M-CSF) through activation of the transcription factors PU.1 and IRF8 (8). We recently showed that the retinoic acid-related orphan receptor (RORC1/RORγ) orchestrates emergency myelopoiesis by suppressing negative (Socs3 and Bcl3) and promoting positive (C/EBPβ) regulators of granulopoiesis, as well as the key transcriptional mediators of myeloid progenitor commitment and differentiation to the monocytic/macrophage lineage (IRF8 and PU.1) (9). Of note, expansion of circulating RORC1^{+} myeloid cells marked advanced cancer-related inflammation and the expansion of immature suppressive cells (9).

In acute inflammation, notably during acute infections, myeloid progenitors expand and differentiate into activated pro-inflammatory monocytes, which eventually migrate into tissues where they differentiate into macrophages and dendritic cells (10, 11). On the other hand, in chronic inflammatory states (e.g., cancer, chronic infection and autoimmune disease) the differentiation of myeloid progenitors into mature immune cells is impaired, a condition that leads to the expansion and accumulation of a population of immature myeloid cells named myeloid-derived suppressor cells (MDSCs) (12). MDSCs consist of a heterogeneous population characterized by high plasticity and strong capacity to reduce cytotoxic functions of T and NK cells (13). MDSCs are conventionally divided into 2 subsets, monocytic (M-MDSCs) and granulocytic (PMN-MDSCs), based on the expression of specific markers that differ among human and mouse cells. In humans, the M-MDSC and PMN-MDCS subsets are defined as CD11b^{+}CD14^{+}HLA-DR$^{-/low}$CD15^{-} and CD11b^{+}CD14^{-}CD15^{+}HLA-DR$^{low/-}$, respectively, while their corresponding murine subsets are indicated as CD11b^{+}Ly6C^{high}Ly6G^{-} and CD11b^{+}Ly6C^{low}Ly6G^{+} cells (11).

From a biochemical and functional perspective, suppressive PMN-MDSCs are characterized by the production of reactive oxygen species (ROS) and arginase 1 (Arg1), whereas M-MDSCs predominantly express the inducible nitric oxide synthase (iNOS) gene and produce nitric oxide (NO). Both pathways promote depletion of the amino acid l-arginine and down-regulation of T cell receptor (TCR) ζ-chain expression, consequently leading to cell cycle arrest (14). Combined production of ROS and NO results in peroxynitration of TCR and promotes T cell apoptosis (15). Additionally, expression of indoleamine 2,3 dioxygenase (IDO) (16), cyclooxygenase (COX1) (17) and the programmed death-ligand 1 (PD-L1) (18) by activated MDSCs concur to immune suppression. MDSCs further promote T regulatory (Treg) cell expansion to prevent anti-tumor T cell effector functions (11, 19–21). A recent meta-analysis performed on a cohort of 1864 patients evaluated the prognostic value of MDSCs in various types of cancers and concluded that their elevated frequency is associated with shorter overall survival (OS) and poor disease-free survival/recurrence-free survival (DFS/RFS) (22). Based on their critical pro-tumor role, efforts are underway to define strategies that can reprogram or functionally deplete MDSCs in order to evaluate their antitumor efficacy alone or in combination with anti-checkpoint inhibitors (ICIs) (23). Since persistent immunological stress promotes the pathological differentiation of myeloid cells, MDSC expansion has been reported also in autoimmune diseases (AD) (12). Similarly, in some infections, caused either by bacteria (e.g., *M. tuberculosis*, *Staphylococcus aureus*) or viruses (e.g., hepatitis B virus/HBV, hepatitis C virus/HCV, human immunodeficiency viruses/HIV), the host's immune response is not able to remove the pathogen, which instead persists and leads to a chronic inflammatory state. In these pathological conditions, the accumulation of M-MDSCs is stimulated to restrict T cell effector functions and to recruit Treg cells in order to resolve inflammation and re-establish immune homeostasis (24, 25). In infections, pathogen recognition by innate immune receptors (e.g., Toll-like receptor), other than cytokines and growth factors, is the key event responsible for M-MDSCs expansion (25).

Targeting MDSCs appears to provide a specular perspective in cancer vs. autoimmune conditions. Here we discuss the role of MDSCs in cancer and autoimmune diseases, highlighting their main suppressor mechanisms and possible therapeutic interventions.

THE IMMUNOSUPPRESSIVE ARMAMENT OF MDSCS AND ITS IMPACT IN CANCER

Beyond being highly heterogeneous, MDSCs are also highly plastic (26), therefore the surrounding microenvironment shapes MDSCs' functions to suppress immune responses through multiple mechanisms (**Figure 1**), including depletion of metabolites critical for T cell functions, expression of immune checkpoint inhibitors, secretion of immunosuppressive molecules, production of reactive oxygen and nitrogen species and regulation of lymphocyte homing.

Depletion of Metabolites Critical for T Cell Functions

A metabolic feature of MDSCs is the up-regulation of enzymes/transporters that pauperize essential amino acids from the extracellular space. This results in both microenvironmental depletion of essential nutrients for T cells and in the generation of molecules endowed with immunomodulatory activities (e.g., nitric oxide, polyamines and kynurenines). Cysteine is an example of an amino acid that T cells cannot produce either by intracellular conversion of methionine or by import of extracellular oxidized cysteine (27). Usually, antigen-presenting cells couple the import of extracellular oxidized cysteine with the export of cysteine, thereby creating a circuit of symbiotic nutrients sharing that feeds T cell activation. In contrast, MDSCs up-take cystine through the xc- transporter but do not export cysteine, thus limiting the extracellular pool of cysteine required for T cell activation (28). MDSCs express copious amount of IDO1 that converts tryptophan in kinurenines inducing Treg cells expansion (29), dampening dendritic cell immunogenicity (30) and concomitantly depriving T cells of an essential nutrient (31). Several preclinical studies have demonstrated the therapeutic potential of IDO inhibition in combination with both

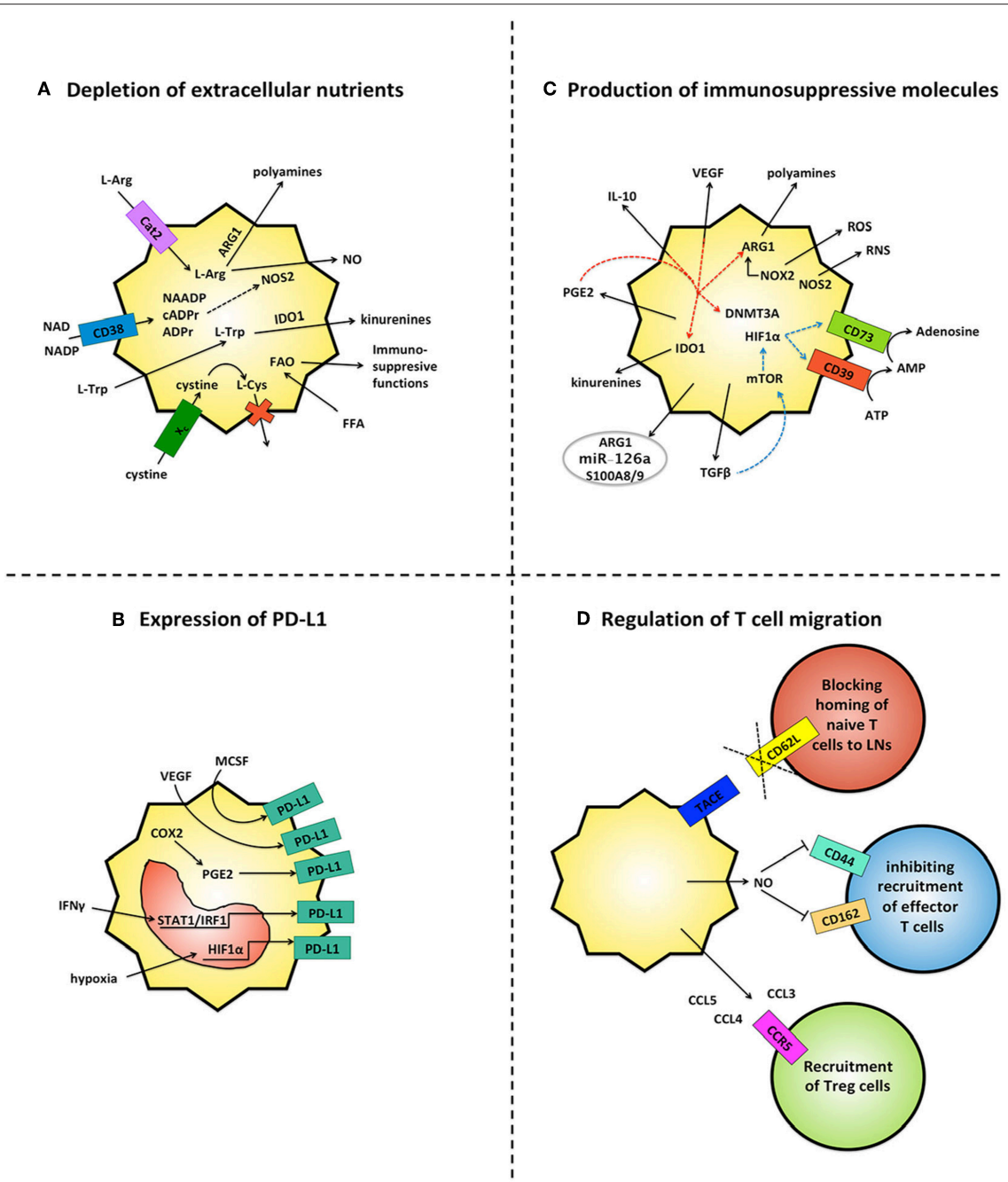

FIGURE 1 | MDSCs inhibit immune responses by multiple mechanisms. **(A)** MDSCs deplete the extracellular microenvironment of essential nutrients for T cells. Through the up-regulation of metabolic enzymes (e.g. ARG1, NOS2, IDO1) and ectoenzymes (e.g. CD38) MDSCs consume copious amounts of amino acids (L-Arg, L-Trp) and NAD, and concomitantly produce molecules endowed with immunomodulatory activities (e.g. nitric oxide/NO, polyamines and kinurenines). Further, MDSCs internalize cystine without releasing the oxidized L-Cys and up-take of FFA, which fuels FAO and expression of immunosuppressive activities. **(B)** MDSCs up-regulate PD-L1 in response to multiple microenvironmental signals, including hypoxia via HIF1α, IFNγ via STAT1/IRF1, MCSF and VEGF via unknown mechanisms. Up-regulation of COX2 and PGE2 are also found associated with PD-L1 expression. **(C)** MDSCs release a range of immunosuppressive soluble molecules. They produce ROS and RNS through NOX-2 and NOS2, adenosine via CD39 and CD73, kinurenines via IDO1, polyamines via ARG1, anti-inflammatory cytokines (IL-10, TGFβ) and PGE2. Both TGFβ (blue lines) and PGE2 (red lines) also create autocrine loops that sustain the production of additional suppressive molecules. TGFβ induces the ectoenzymes CD39 and CD73 via HIF-1 α and PGE2 promotes expression of immunosuppressive molecules (IDO1, IL-10, ARG1 and VEGF) as well as repression of immunogenic-associated genes via DNMT3A. MDSCs also secrete exosomes which contain different molecules, such as immunosuppressive ARG1, inflammatory S100A8/9 and the oncogenic miR-126a. **(D)** MDSCs modulate T cell trafficking. They limit homing of naïve T cells to LNs by TACE-mediated cleavage of CD62L on T cells and they impair extravasation of effector T cells through NO-mediated down-regulation of adhesion molecules CD162 and CD44. In contrast MDSCs support the recruitment of $CCR5^{+}$ Treg cells by production of CCL3, CCL4, CCL5.

chemotherapy and immune checkpoint blockers. Accordingly, phase II/III human trials will evaluate two small molecule enzyme inhibitors of IDO1 (epacadostat and GDC-0919/navoximod) in human cancer patients (32).

Metabolic conversion of L-arginine (L-Arg) through either iNOS or Arg1 is the first and the main mechanism associated with the immunosuppressive activities of MDSCs. In addition, Arg1 supports tumor cell proliferation by producing ornithine and polyamines, whereas iNOS promotes T cell death through NO generation and consequent tyrosine nitration and S-cysteine nitrosation of various proteins (33, 34). Strikingly, a recent paper reported that bone marrow (BM)-derived MDSCs require direct cell-cell contact rather than Arg1 expression or production of soluble factors to mediate immunosuppression in different tumor models (e. g. melanoma, colon carcinoma and lymphoma) (35).

The expression of Arg1 and iNOS differs among mouse and human myeloid cells, with the former predominantly expressed by the granulocytic subset and the latter by the monocytic counterpart (36). Preclinical studies and clinical trials with inhibitors of phosphodiesterase-5, (e.g., sildenafil and tadalafil) pointed out that a reduction of both iNOS and Arg1 activities in MDSC reactivates antitumor immunity (37–39).

Nicotinamide adenine dinucleotide (NAD) is one of the most important coenzymes in mammalian metabolic pathways (40). CD38 is an ectoenzyme that, by consuming extracellular NAD, leads to mitochondrial dysfunction of surrounding cells, as observed in metabolic diseases and cancer (41). CD38 was found up-regulated in MDSCs from various preclinical tumor models and cancer patients (neck cancer and non-small cell lung cancer). Along with the detrimental effects associated with depletion of microenvironmental NAD, CD38 generates second messengers associated with calcium signaling (42), resulting in an increased amount of NO that favors tumor growth (43). Treatment of multiple myeloma patients with daratumumab (an antibody directed toward CD38) was associated with reduction of PMN-MDSCs, suggesting that this event might contribute to the therapeutic effect of anti-CD38 (44). Beyond being a key regulator of energy metabolism and ATP production, NAD is the substrate for numerous NAD-consuming enzymes that participate in cell signaling, including mono- and poly-(ADP-ribose) polymerases, sirtuins (SIRT) and CD38/CD157 (45).

Interestingly, in different tumor models (i.e., thymoma and melanoma) the lack of SIRT1 in MDSCs fuels the glycolytic pathway through the mTOR-HIF-1α pathway. This metabolic reprogramming is associated with a functional switch of immunosuppressive MDSCs toward a pro-inflammatory (NO, TNF, IL-12) and anti-tumor phenotype (46). Additional studies confirmed the importance of metabolic pathways on MDSC activity. In both tumor bearing mice and humans, tumor-derived cytokines induce expression of cell surface lipid transport receptors on MDSCs via STAT3 and STAT5 (47). This results in increased fatty acid uptake and oxidative metabolism in association with activation of MDSCs' immunosuppressive mechanisms. Therefore, hampering the intracellular accumulation of lipids (47) as well as pharmacological inhibition of FAO (48) blocks the immunosuppressive functions of MDSCs, improving the efficacy of either immunotherapy or low-dose chemotherapy.

Expression of Immune Checkpoint Inhibitors

It is not surprising that several pre-clinical and clinical studies have found an association between PD-L1 expression by MDSCs and immunosuppression (49). Mechanistically, the expression of PD-L1 on MDSCs can be triggered through different pathways whose relative importance may depend on different microenvironmental features of tumor regions as well as on the type of tumor. For example, hypoxia induces PD-L1 expression on MDSCs via HIF-1α (18). In line with this, the blockade of PD-L1 expression under hypoxia enables MDSCs to support T cell activation; therefore, the combination of PD-L1 neutralization with HIF-1α inhibitors could improve the clinical response of patients with advanced disease. In a preclinical model of colitis-associated colorectal cancer, PD-L1 emerged to be mainly expressed by tumor-infiltrating M-MDSCs ($CD11b^{+}Ly6C^{+}$ cells) in response to IFNγ via STAT1-IRF1 axis (49). M-CSF and VEGFA produced by human liver cancer cell lines can induce PD-L1 expression on immature myeloid cells ($CD33^{Dim}HLA\text{-}DR^{-}$ cells) isolated from peripheral blood of healthy donors. Accordingly, circulating $PD\text{-}L1^{+}$MDSCs were detected in HCC patients and their frequency increased with disease progression, although it did not correlate with serum concentration of M-CSF or VEGF (50).

In mouse bladder cancers, PD-L1 expression on tumor-associated myeloid cells is associated with the expression of cyclooxygenase-2 (COX2), microsomal prostaglandin E synthase-1 (mPGES1), prostaglandin E2 (PGE2) and the capacity to induce apoptosis of $CD8^{+}$ T cells (51). Either genetic or pharmacological inhibition of PGE2 restrained tumor-induced PD-L1 expression on myeloid cells. PGE2 can also directly and indirectly blunt the activation of CD8 T cells (52). Further, PGE2 has been shown to promote MDSCs activity by inducing up-regulation of additional immunosuppressive molecules (e.g., IDO, IL-10, ARG-1, and VEGF) (53–55), as well as by repressing immunogenic-associated genes via DNA methyltransferase 3A (DNMT3A) (56). In agreement, human MDSCs from ovarian cancer patients display a similar hypermethylation signature in connection with PGE2-dependent DNMT3A overexpression (56). Recently, in a pre-clinical model of colorectal cancer there has emerged a circuit based on down-regulation of receptor-interacting serine/threonine-protein kinase 3 (RIPK3) in MDSCs linked to the production of PGE2. This autocrine loop is crucial for MDSC accumulation and immunosuppressive activity and the consequent promotion of colon carcinogenesis (57). Therefore, PGE2 represents a very attractive drugable target that can be exploited to modulate MDSCs' immunosuppressive functions in multiple contexts.

Interestingly, not only are high levels of circulating MDSCs ($CD33^{+}CD11b^{+}HLA\text{-}DR^{-}$cells) predictive of a poor response of advanced melanoma patients to ipilimumab (anti-CTLA4) therapy (58), but circulating MDSCs of non-responders showed higher expression of PD-L1 by PMN-MDSCs and copious

production of NO by M-MDSCs (59). In line with this, in models of lung and renal cell carcinoma, entinostat, a class I histone deacetylase inhibitor, improved the anti-tumor effect of anti-PD-1 antibodies by reducing the expression of Arg1, iNOS, and COX2 in MDSCs (60). Therefore, different clinical trials are studying the combination of entinostat with immune checkpoint blockade (ICB) in patients with renal cell carcinoma and other advanced solid tumors (61).

Production of Immunosuppressive Molecules

MDSCs express high levels of Ectonucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, CD39) and the ecto-5'-nucleotidase, which convert the extracellular ATP released by dying cells in adenosine. Extracellular adenosine is a powerful immunosuppressive factor that impairs differentiation of naïve $CD8^+$ T cells in effector cells (62), inhibits cytolitic activity of NK and activated T cells (63), and it promotes the immunosuppressive functions of tumor-associated macrophages (TAM) and expansion of PMN-MDSCs (64). MDSCs also produce copious amounts of immunosuppressive cytokines, such as TGF-β and IL-10, which induce the generation of Treg cells, differentiation of pro-tumoral IL-12^{low} TAM and direct suppressive effects on T effector cells (65, 66). TGF-β can also exert either promoting or inhibiting effects on MDSCs themselves (67, 68). Exposure of murine BM-derived MDSC or healthy human PBMCs to TGF-β, along with conditioned medium of either MEER (murine pharyngeal epithelial cells expressing HPV16 E6 and E7, and hRas) or human head and neck squamous cell carcinoma cells (SCC-47), triggered $CD11b^+Gr1^+$ MDSCs to acquire antigen-presenting capability and Fas-dependent tumor cells killing activity (68). Consequently, in mice transplanted with MEER tumor, the combination of radiotherapy with intra-tumoral adoptive transfer of TGF-β-conditioned MDSCs resulted in a durable tumor clearance (68). In apparent contrast, *ex vivo* studies indicate that TGF-β skews differentiation of human peripheral blood $CD14^+$ monocytes toward immunosuppressive M-MDSCs (67). Accordingly, in mouse models of lung and mammary carcinoma, disruption of TGF-β signaling in myeloid cells resulted in decreased expression of CD39 and CD73, in association with increased infiltration of T lymphocytes, reduced density of blood vessels and diminished tumor progression (69). A recent study highlighted that the frequency of $CD39^+CD73^+$ MDSCs in the NSCLC patients is closely correlated with disease progression and chemotherapeutic resistance (70). Mechanistically, it was confirmed that tumor-derived TGF-β triggers CD39 and CD73 expression on circulating and tumor-infiltrating MDSCs via activation of mTOR/HIF-1α-signaling (70). Along with these findings, diabetic patients with ovarian carcinoma gain beneficial anti-tumor effects by metformin treatment. Indeed, this anti-diabetes drug down-regulates HIF-1α via the activation of the AMP-activated protein kinase α (AMPKα) and consequently decreases expression of CD39 and CD73 on both M- and PMN-MDSCs. Therefore, metformin treatment leads to the reduction of circulating $CD39^+CD73^+$ MDSCs and enhances the anti-tumor activities of circulating $CD8^+$ T cells, promoting longer overall survival of ovarian cancer patients (71). New evidence indicates that MDSCs can secrete exosomes which contain molecules, such as immunosuppressive Arg-1 (72), inflammatory S100A8/9 (73) and the oncogenic miR-126a (74). Interestingly, *in vivo* administration of PMN-MDSCs derived exosomes to DSS-treated mice ameliorates colitis, thereby confirming the immunosuppressive activity of molecules included in the extracellular vesicles (EV) (72). In cancer bearers, tumor cells are the major source of circulating EV. Recently a set of microRNAs (miR-146a, miR-155, miR-125b, miR-100, let-7e, miR-125a, miR-146b, miR-99b) has been identified that are transferred via EV from melanoma cells to circulating monocytes, driving their conversion into MDSCs. Therefore, high levels of plasma MDSC-miRs emerged as valuable predictive peripheral blood biomarkers of resistance to ICB in cancer (75).

Production of ROS

A major mechanism used by PMN-MDSCs to suppress antigen-specific T cells is the secretion of copious amounts of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, hydrogen peroxide and singlet oxygen (34). Accordingly, in a MDSCs/T cells co-culture system, the addition of ROS inhibitor catalase blunts the immunosuppressive effects of MDSCs (76). ROS production by MDSCs is driven by the up-regulation of NADPH oxidase activity, in particular the NOX2 subunits 47 (phox) and gp91 (phox). Indeed, the lack of NOX2 impaired both generation of ROS by MDSCs and their ability to suppress antigen-specific $CD8^+$ T cells (77). In addition, NOX2-dependent ROS production supports MDSC expansion (77) and recruitment in tumors through the up-regulation of VEGF receptors (78). Myeloperoxidase is another ROS-producing enzyme that, along with ARG-1, is more abundantly expressed by PMN-MDSCs than neutrophils, contributing to suppression of antigen-specific T cell responses in tumor bearers (79).

MDSCs survive despite elevated levels and continuous production of ROS through the expression of the Nrf2 transcription factor, an important mediator of the cellular antioxidant response (80). Indeed, genetic ablation of Nrf2 impaired generation, survival and suppressive potency of MDSCs in models of mammary and colon tumor (80). To counteract the detrimental effects of oxidative stress, MDSCs up-regulate their anaerobic metabolism (i.e., glycolysis), which leads to the intracellular accumulation of the anti-oxidative intermediate phosphoenolpyruvate (81). Overall, targeting redox-regulation of MDSCs is emerging as a promising therapeutic opportunity in multiple diseases, such as cancer, infection, inflammation, and autoimmune disorders (82).

Regulation of Lymphocyte Homing

MDSCs impair T cell activation also by inhibiting the homing of naïve $CD4^+$ and $CD8^+$ T cells to lymph node (83). This effect is dependent on down-regulation of CD62L on naïve T cells through the expression of TNF-α-converting enzyme (TACE/ADAM17) by MDSCs (84). Growing evidence suggests that this ability of MDSCs to hinder T cell activation plays a

crucial role in establishing maternal–fetal tolerance in pregnant mice and women (85). An expansion of MDSCs in maternal peripheral blood occurs during human pregnancy, and higher frequency of circulating PMN-MDSCs has recently emerged as a favorable predictor of the success rate of *in vitro* fertilization treatment (86). In addition, MDSCs can hamper the recruitment of circulating effector T cells into tissues by inhibiting the expression of CD162, a ligand of P-selectin and CD44, the receptor for the extracellular matrix component hyaluronic acid (HA) (87). The block of effector T cell homing is paralleled with the recruitment of immunosuppressive T cells. For example, in two mouse models of melanoma, tumor M-MDSCs produce CCL3, CCL4 and CCL5 which drive the recruitment of $CCR5^+$ Treg cells (88).

MDSCS IN AUTOIMMUNITY

Autoimmunity is defined as an immune response against self-antigen. The tolerance against self-antigens is a tightly regulated process that involves both innate and adaptive immunity and implies the possibility to eliminate or inhibit self-reactive lymphocytes. In autoimmune diseases (AD), both genetic and environmental factors contribute to the breakdown of tolerance (89), which results in the generation of auto-reactive B and T cells. Clinical manifestation of AD derives from tissue damage caused by self-reactive T cells. In contrast to their deleterious role in tumors, MDSCs have been studied in various models of AD to evaluate potential beneficial role (90).

Due to their prevalent immunoregulatory phenotype MDSCs represent an important cell population that can be therapeutically used to suppress T cell functions. On this line, new work indicates their accumulation in secondary lymphoid organs of patients with autoimmune disorders, including type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and autoimmune hepatitis (91), and a number of studies have provided insight into the use of MDSCs for treatment of AD (12).

Autoimmune Diabetes

Type 1 diabetes (T1D) is among the most prevalent autoimmune diseases worldwide, affecting ∼10–20 million people. The disease occurs as a consequence of a disruption in immune-regulation, resulting in the expansion of autoreactive CD4 and CD8 T cells and autoantibody-producing B lymphocytes (92), which leads to the destruction of pancreatic insulin-producing β-cells in the pancreas (93). Both $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells can transfer autoimmune diabetes to immunodeficient hosts in mouse models (94, 95), and T cells are found in inflammatory infiltrates surrounding pancreatic islets in T1D patients (96).

Rising evidence of MDSCs' involvement in the pathogenesis of T1D opens new potential therapeutic strategies for T1D. In two different murine models, Yin et al. provided evidence that adoptive transfer of MDSCs against autoreactive T cells prevented pancreatic islets damage (97). Furthermore, it was shown in NOD/SCID mice that temporary B cell depletion induced expansion of regulatory $CD11b^+Gr1^+$ cells, which directly suppress diabetogenic splenocytic T cell functions in an IL-10-, NO-, cell contact-dependent manner (98).

It has also been shown that the contribution of the C3 complement factor to the development of autoimmune diabetes depends directly on MDSCs. In fact, the C3 deficiency in the Streptozotocin-induced diabetes (STZ) model produces an increase in the frequency of MDSCs and enhances their ability to suppress the proliferation of diabetogenic T cells through arginase/iNOS activity (99).

A paradoxical increase in the frequency of MDSCs was reported in the peripheral blood of T1D patients, as well as in the peripheral blood and secondary lymphoid organs of diabetic NOD mice (100). Of note, this increased frequency of MDSC was counterbalanced by the decreased MDSC number within the pancreatic microenvironment of diabetic NOD mice, suggesting that the lack of islet MDSCs may favor autoimmune diabetes development (100).

A strong association has been demonstrated between polymorphisms of NOD-like receptor family-pyrin domain containing 3 (NLRP3) and predisposition to the T1D. Carlos et al. showed that the ablation of NLR3P in both NOD and STZ-treated diabetic mice, as a consequence of elevated IL-6 expression, produced an expansion of MDSCs in pancreatic lymph nodes (PNLs), which inhibits the inflammatory T cells response in the pancreatic islets and prevents the onset of T1D (101). This evidence proposes the expansion of MDSCs as strategy for dampening the autoimmune T cell response and preventing T1D.

Multiple Sclerosis (MS)

MS is an autoimmune inflammatory demyelinating disease and a prime cause of neurological disability in young adults (102). Clinically, MS manifests itself as neurological deficits that frequently exhibit a relapsing and remitting pattern (RRMS) reflecting the characteristic recurrent bouts of T cell-mediated attack upon antigens in neuronal myelin sheaths. MS can resolve completely or leave residual deficits of any grade (102).

Experimental Autoimmune Encephalomyelitis (EAE) is the most used animal model of autoimmune inflammatory diseases of the central nervous system (CNS), and it resembles MS. Active EAE is induced by immunization with CNS tissue or myelin peptides, such as myelin basic protein (MBP) and proteolipid protein (PLP) emulsified in various adjuvants, usually containing bacterial components highly capable of activating the innate immune system via pattern recognition receptors (i.e., complete Freund's adjuvant, CFA) (103). This leads to the peripheral activation of myelin-specific T cells which are subsequently recruited together with myeloid cells in the CNS. These provoke the release of inflammatory cytokines and chemokines, producing demyelination and CNS damage (103, 104).

In the last decade, the presence and the activation state of MDSC subsets in MS have been objects of intense investigation. In a model of experimental autoimmune encephalomyelitis (EAE), Zhu et al. first characterized the subsets of accumulating myeloid cells in blood, spleen and CNS. They showed that a small population of $CD11b^+Ly6C^{hi}$ immature monocytic cells could exert the potent suppression of both $CD4^+$ T cells and $CD8^+$

T cells *ex vivo*, inducing their apoptosis through nitric oxide production (105).

In contrast, two different and independent works highlighted a more pro-inflammatory and pathogenetic role of the $CD11b^{+}Ly6C^{hi}$ cell subset. Mildner et al. proposed that CCR2-expressing $CD11b^{+}Ly6C^{hi}$ monocytes are indispensable for the pathogenesis of MS due their capability to express MHC class II molecules and inflammatory cytokines, which would support local autoimmune encephalitogenic T cell activation (106).

King et al. instead proposed a dynamic interpretation of the role of $CD11b^{+}Ly6C^{hi}$ cells in MS. $CD11b^{+}Ly6C^{hi}$ cells that accumulate in the blood and CNS of mice immunized by myelin, before the onset of clinical episodes, would behave like inflammatory monocytes rather than MDSCs. Next, the CNS microenvironment would evolve during the course of the disease, inducing a more suppressive and anti-inflammatory phenotype of $CD11b^{+}Ly6C^{hi}$ immediately before the onset of the remission phase (107). In line with this, the distribution of protective Arg1-expressing MDSCs within the spinal cord of EAE mice was confirmed during the remitting phase (108).

A pivotal role in the regulation of CNS autoimmune inflammation was provided also for PMN-MDSCs, which accumulate in the peripheral draining lymph nodes (LNs) and in the spinal cord of EAE-immunized mice, prior to the remission phase of the disease (109). Moreover, granulocytic $CD33^{+}CD15^{+}$ MDSCs were significantly enriched in the peripheral blood of subjects with active MS (109). Noteworthily, in EAE mice, adoptively transferred PMN-MDSCs ameliorated the disease and delayed its onset through the significantly reduced expansion of autoreactive T cells in the draining of LNs (109).

The initiation and severity of the chronic disease phase in MS is associated with the accumulation of these B cell aggregates. Knier et al. showed that the frequency of $CD138^{+}$ B cells in the cerebrospinal fluid (CSF) of human patients with MS was negatively correlated with the frequency of PMN-MDSCs in the CSF (110).

Analyses of the dynamic of immune cell populations in the CSF and CNS parenchyma of mice during EAE revealed a persistent population of $Ly6G^{+}$ cells recruited to the CSF space at the beginning of the recovery stage. Cantoni et al. have recently identified that the decreased number of blood M-MDSCs in relapsing MS patients is associated with increased MDSC expression of miR-223 compared to healthy subjects, and it is accompanied by a reduced expression of STAT3 and Arg1 (111, 112). These data are corroborated by the evidence that miR-223 deficient mice showed reduced EAE severity and pathology progression as result of an increase in MDSC number in the spleen and CNS (112).

Rheumatoid Arthritis (RA)

RA is a systemic AD characterized by a chronic synovitis that results from the sustained influx of various leukocyte populations into the synovial space, thereby leading to destruction of the joint cartilage and erosion of bone (113). $CD4^{+}$ T cells, and the cytokine milieu within the affected joints, are critically implicated in the pathogenesis of RA, as they promote differentiation toward pro- and anti-inflammatory T cell subpopulations, including Th1, Th2, Th17, and Treg cells (114). Elevated levels of pro-inflammatory Th17 cells as well as defects in anti-inflammatory Treg cells have been reported in RA patients (115, 116) and in experimental arthritis in mice (117, 118), but the mechanisms governing the imbalance of Th17/Treg cells resulting in RA remain unclear. Discordant results regarding the effect of MDSCs on RA have been reported in both preclinical mouse models and patients (119). Jiao et al. reported that both the prevalence of circulating MDSCs and plasma Arg1 increased significantly in RA patients compared to healthy controls and were negatively correlated with peripheral Th17 cells (116). Unfortunately, these MDSC-like cells were defined only by phenotypic marker expression, and the suppressive properties of these cells toward T cells were not tested in that study. A beneficial accumulation of MDSCs, mainly PMN-MDSCs, was reported in the spleens of arthritic DAB/1 mice with collagen-induced arthritis (CIA) at the peak of the disease (35 days after CIA induction), and these cells prevented both the proliferation of $CD4^{+}$ T cells and their differentiation into Th17 cells *in vitro*, via an Arg1-dependent mechanism (120). Moreover, *in vivo* depletion of PMN-MDSCs with anti-Gr1 mAb delayed the spontaneous resolution of joint inflammation in mice with CIA, while adoptive transfer of $CD11b^{+}Gr1^{+}$ MDSCs reduced the severity of CIA *in vivo* and decreased the number of total $CD4^{+}$ T cells and Th17 cells in the dLN (120). It has also been demonstrated that PMN-MDSCs in synovial fluid (SF) from mice with proteoglycan (PG)-induced arthritis (PGIA) could potently suppress autoreactive T cell proliferation and dendritic cell maturation (121). Recently, the adoptive transfer using three types of splenic MDSCs (total MDSCs, M-MDSCs and PMN-MDSCs) obtained from CIA mice demonstrated that all these kinds of MDSCs markedly ameliorated inflammatory arthritis and profoundly inhibited T cell proliferation (122). All the aforementioned studies revealed promising therapeutic effects of MDSCs in an animal model of RA (120–122). However, a few recent papers have shown that MDSCs can aggravate inflammatory arthritis in mice (123–125). Such a discrepancy in the results could be due to the heterogeneity of MDSCs, inflammatory context-dependent interaction between MDSCs and different subsets of $CD4^{+}$ T cells and different states of disease.

Systemic Lupus Erythematosus (SLE)

Lately, the possible involvement of MDSCs in SLE, a systemic AD characterized by elevated levels of autoantibodies against nuclear materials (ANAs) and cellular infiltration of various organs (126), has also been addressed. Administration of laquinimod, an immunomodulatory drug currently in clinical trials for MS and lupus nephritis, in a (NZB × NZW)F1 murine model of SLE, delayed lupus manifestation by inducing expansion of M-MDSCs and PMN-MDSCs in the spleen and kidney (127). In addition, IL-33 blockade in MRL/Fas^{lpr} mice could significantly ameliorate the severity of SLE disease, and this therapeutic effect was closely associated with expansion of MDSCs and Treg cells, accompanied by reduced Th17 cells and inflammatory cytokines in the serum and kidneys (128). Another study reported that deletion of

CD24 in a lupus-like disease model (tm24KO mice) led to the expansion of MDSCs and Treg cells that augmented immune tolerance, accompanied with the alleviation of lupus-like pathology (129).

However, the protective role of MDSCs in lupus is challenged by much evidence. A significant increase in HLA-DR^{-}CD11b^{+}CD33^{+} MDSCs, including both CD14^{+}CD66b^{-} monocytic and CD14^{-}CD66b^{+} granulocytic MDSCs, was reported in the peripheral blood of patients with active SLE, and the frequency of these populations positively correlated with serum Arg1 activity, Th17 responses and lupus severity. Moreover, adoptive transfer of non-MDSC-depleted PBMCs from SLE patients in NOD/SCID mice, induced lupus nephritis-like symptoms via Th17 response in an Arg1-dependent manner (130). A critical pathogenic role of MDSCs was recently documented also in lupus nephritis (LN), one of the most severe manifestations of SLE. In particular, in a TLR-7 agonist imiquimod-induced lupus mice model, MDSCs induced severe podocyte injury in the glomeruli of kidneys through increasing ROS, activating p-38MAPK and NF-κB signaling (131). These data infer that changes in both percentage and function of MDSCs could be crucial during SLE development; however, it is still not clarified which factors influence the behavior of MDSCs in the lupus microenvironment.

Inflammatory Bowel Disease (IBD)

In IBD an aberrant homeostasis between intraluminal bacterial antigens and the mucosal immune system leads to chronic inflammatory pathology. IBD encompasses both Crohn's disease (affects any part of the gastrointestinal part) and ulcerative colitis (affects colon and/or rectum) (132, 133). IBD is widely considered to result from an overlay aggressive Th1 immune response and excessive IL-23/Th17 pathway activation, as well as decreased Treg responses (134). Interestingly, an increase in the frequency of human CD14^{+}HLA-DR$^{-/low}$ MDSCs with suppressive properties was observed in the peripheral blood from IBD patients (135, 136). In agreement, hyperactivation of STAT3, a known regulator of MDSC expansion, has been associated recently with protection from experimental colitis (137, 138), while another study reported that the resistance to colitis in gp130$^{757F/F}$ mice occurred via myeloid-specific STAT3 activation, expansion of PMN-MDSCs in the colon and increased production of suppressive cytokines (138). In contrast with these observations, another study showed that adoptively transferred BM Ly6C^{high} cells are recruited into the colon and differentiate into inflammatory DCs and macrophages (139), contributing to intestinal inflammation in a TNFα-dependent manner (140) and triggering proliferation of antigen-specific T cells (141). In addition, a recent paper reported that IBD patients had high peripheral blood levels of CD14^{+}HLA-DR$^{-/low}$ MDSCs, associated with exacerbated IBD (142). Hence, the intrinsic plasticity of MDSCs renders them prone to conversion into effector cells; it is very important to evaluate how their suppressive potential can be harnessed therapeutically to benefit IBD patients.

Others (Myastenia, Psoriasis, Uveitis, Trombocytopenia)

Additional evidence supporting the immune regulation capabilities of myeloid cells in ADs came from a mouse model of myasthenia gravis, by which McIntosh and Drachman showed a population of "large suppressive macrophages" (LSM) capable of suppressing T cell proliferation (143). A counterintuitive role of MDSCs is emerging in psoriasis. Psoriatic patients display an increased frequency of granulocytic and monocytic MDSC subtypes in blood and skin compared to healthy subjects (144–146). Lauren et al. highlighted a high heterogeneity of MDSCs in this pathology in terms of a diverse capability to inhibit allogeneic T cells through the use of either the IL-17/Arg-1 or IFNγ/iNOS axis as suppressor mechanisms (144). Furthermore, these cells are capable of producing various molecules, including matrix metalloproteinase-9 and−1, interleukin-8, growth-related oncogene, and monocyte chemoattractant protein 1, which could contribute to further establishing a pro-inflammatory immune response and confer less immunosuppressive attitudes to MDSCs (145). Soler et al. showed that psoriatic M-MDSCs directly suppressed CD8^{+} T-cell proliferation less efficiently than healthy control M-MDSCs (146). Kerr et al. have also described a dynamic presence of MDSCs in the inflamed eye of autoimmune uveoretinitis (EAU) subjects. In this model, MDSCs isolated from the inflamed eye were able to profoundly suppress T cell proliferation (147). In another study, this group showed an infiltrating subset of CD11b^{+}Gr1^{+}Ly6C^{+} cells which suppressed the T cell mediated pro-inflammatory response in a TNF receptor 1-dependent manner (148). Finally, Hou et al. have described impaired numbers and suppressive functions of MDSCs in the blood and spleens of adult patients with primary immune thrombocytopenia (ITP), where cell-mediated immune responses are involved in platelet destruction (149). The overall scenario indicates that MDSC manipulation may provide therapeutic benefit during the course of autoimmune disorders.

Perspectives of MDSC Reprogramming in Therapy

The gold-standard treatment for autoimmune diseases relies on immunosuppressive drugs because of their high effectiveness in ameliorating symptoms in many patients. However, long-term and high-dose administration of such drugs can lead to life-threatening, opportunistic infections and long-term risk of malignancy (150). Furthermore, the generation of new therapeutic approaches exploiting the CTLA-4-mediated costimulatory blockade (151–154) or the neutralization of pro-inflammatory cytokines (155) frequently result in increased side effects and lack of responsiveness in long-term administration. In this scenario, cell-based therapy that exploits the *ex vivo* generation of MDSCs represents an interesting perspective for the treatment of ADs. Indeed, compelling evidence from animal models has provided insights into the potential therapeutic effects of MDSCs adoptive transfer in ADs.

In a murine model of arthritis, BM progenitor cells of healthy mice cultured with a combination of IL-6, G-CSF and GM-CSF became enriched in MDSC-like cells that potently inhibited

antigen-specific and polyclonal T-cells proliferation *in vitro* via the production of nitric oxide. The injection of BM-MDSCs into mice with PGIA ameliorated arthritis and reduced PG-specific T cell responses and serum antibody levels (156). Moreover, addition of tofacitinib (a small-molecule JAK inhibitor currently considered as novel therapy of RA) facilitated the *in vitro* expansion of MDSCs inhibiting their differentiation to DCs, and their adoptive transfer in SKG arthritic mice reduced the severity of the disease (157). A therapeutic effect of BM-derived MDSCs was demonstrated also in a model of SLE. Intravenous injection of MDSCs, differentiated from BM cells of C57BL/6 mice upon stimulation with M-CSF and GM-CSF, induced expansion of Breg cells via iNOS and ameliorated autoimmunity in Roquin$^{san/san}$ lupus mice (158). In another study, BM cells were isolated from wt mice and cultured in the presence of GM-CSF and HSCs, resulting in the generation of MDSCs. Adoptive transfer of these MDSCs in mice with colitis, induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS), decreased intestinal inflammation as well as the levels of IFNγ, IL-17 and TNFα (159). Likewise, in the study by Su et al., which investigated the role of MDSCs in the model of TNBS-induced colitis, transplantation of GM-CSF-induced MDSCs ameliorated intestinal inflammation and downregulated the levels of proinflammatory cytokines (160). In the STZ-treated diabetic mice, Hsieh et al. provided proof that the adoptive transfer of MDSCs, obtained from BM cells cultured *in vitro* with GM-CSF, IL-1β, and IL-6 under normoglycemic conditions, substantially reduced fibronectin accumulation in the renal glomerulus, ameliorating diabetic nephropathy (161). In a mouse model of alopecia areata (AA), it was recently demonstrated that MDSCs can efficiently exert their activity not only through cell-cell contacts or soluble factors, but also by their capability to secret exosomes (Exo) (162). Indeed, Zöller et al. showed that treatment with MDSC-derived Exo from naïve mice prevented the progression of the disease and induced partial hair growth as a result of the inactivation of pro-inflammatory T cells and promotion of T regulatory cell differentiation (163).

Other potential opportunities of MDSC-mediated cell therapy apply to allogeneic transplantation. In this regard, Highfill et al. showed that addition of IL-13 in BM cells cultured with GM-CSF and G-CSF resulted in the production of suppressive MDSCs that efficiently inhibited allo-immune rejection (164). In pancreatic islet transplantation, Chou et al. observed that the presence of small amounts of Hepatic stellate cells (HpSC) into DC culture (BM-cells stimulated for 5 days with GM-CSF) produced a large number of MDSCs that efficiently protected islet allografts (165). Importantly, in this model of allograft transplantation, as well as in transplantation of male skin onto female recipients, it was found that only long-term and multiple injections of MDSCs significantly improved the acceptance of the graft (166). This may be due to the observation that in absence of chronic inflammation MDSCs may terminally differentiate

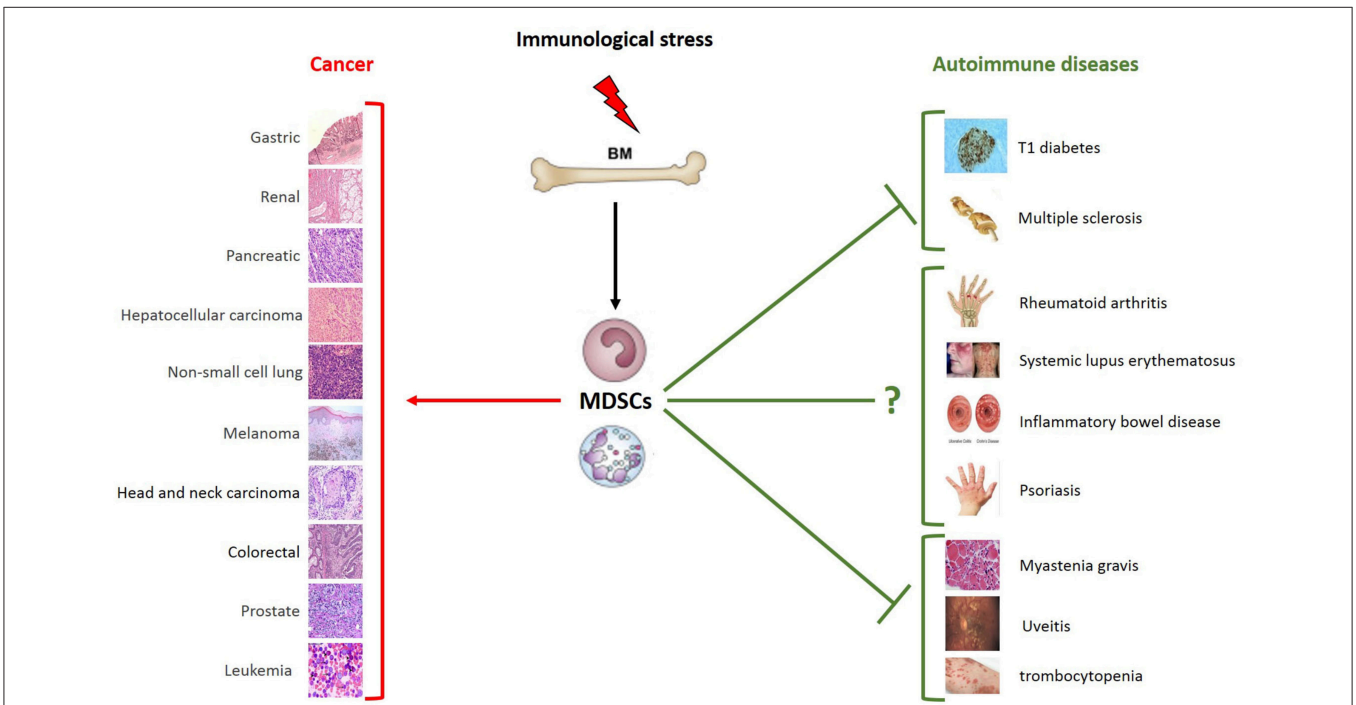

FIGURE 2 | Schematic role of MDSCs in pathology. Immunological stress induces the expansion of MDSCs that play different roles depending on distinct pathological and microenvironmental contexts. MDSCs are characterized by the strong ability to suppress T cell functions. Much clinical and preclinical evidence demonstrates their ability to promote tumor growth and metastasis formation. Given the immunosuppressive phenotype, MDSCs can also play a beneficial role in autoimmune diseases. As shown in the figure, the expansion of MDSCs is protective in some autoimmune diseases, such as type 1 diabetes, multiple sclerosis, myastenia gravis, uveitis and trombocytopenia. Their role in systemic lupus erythematosus, inflammatory bowel disease psoriasis and rheumatoid arthritis remains to be further clarified. The types of tumor and autoimmune diseases in which an expansion of MSDCs have been reported are summarized in the figure. MDSCs, myeloid-derived suppressor cells; BM, bone marrow.

toward a pro-inflammatory phenotype (167, 168). This evidence highlights the need to identify new strategies that stabilize the suppressive phenotype of MDSCs. In this regard, Greifenberg et al. showed that BM-MDSCs differentiated in the presence of LPS and IFN-γ expressed a stable suppressive phenotype (169). Therefore, although many open questions on the therapeutic use of MDSCs remain to be clarified, an increasing number of observations indicate that these cells can potentially be used to control autoimmune diseases and allograft rejection.

DISCUSSION

MDSCs violently emerge in pathological conditions in an attempt to limit potentially harmful immune and inflammatory responses. Mechanisms supporting their expansion and survival are deeply investigated in cancer, in the perspective to reactivate specific antitumor responses and prevent their contribution to disease evolution. These findings will likely contribute to improve the targeting of MDSCs in anticancer immunotherapies, either alone or in combination with immune checkpoint inhibitors. New evidence indicates that the expansion of myeloid cell differentiation in pathology is subject to fine-tuning, as its alterations may support either immunosuppression or autoimmunity. This pathological plasticity is supported by evidence indicating that common MDSC-associated targets may be specularly targeted in autoimmunity vs. cancer (12), and there is now hope that understanding autoimmune mechanisms might serve as a lesson for the development of new anticancer therapies. The functional plasticity and therapeutic ductility of these cells (**Figure 2**) suggest that while MDSC inhibition might succeed as anticancer treatment, their induction is expected to provide therapeutic benefit in autoimmune diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Ueha S, Shand FHW, Matsushima K. Myeloid cell population dynamics in healthy and tumor-bearing mice. *Int Immunopharmacol.* (2011) 11:783–8. doi: 10.1016/j.intimp.2011.03.003
2. Velten L, Haas SF, Raffel S, Blaszkiewicz S, Islam S, Hennig BP, et al. Human haematopoietic stem cell lineage commitment is a continuous process. *Nat Cell Biol.* (2017) 19:271–81. doi: 10.1038/ncb3493
3. Hirai H, Zhang P, Dayaram T, Hetherington CJ, Mizuno SI, Imanishi J, et al. C/EBPβ is required for "emergency" granulopoiesis. *Nat Immunol.* (2006) 7:732–9. doi: 10.1038/ni1354
4. Akagi T, Saitoh T, O'Kelly J, Akira S, Gombart AF, Koeffler HP. Impaired response to GM-CSF and G-CSF, and enhanced apoptosis in C/EBP2-deficient hematopoietic cells. *Blood.* (2008) 111:2999–3004. doi: 10.1182/blood-2007-04-087213
5. Zhang H, Nguyen-Jackson H, Panopoulos AD, Li HS, Murray PJ, Watowich SS. STAT3 controls myeloid progenitor growth during emergency granulopoiesis. *Blood.* (2010) 116:2462–71. doi: 10.1182/blood-2009-12-259630
6. Liu B, Tan W, Barsoum A, Gu X, Chen K, Huang W, et al. IL-17 is a potent synergistic factor with GM-CSF in mice in stimulating myelopoiesis, dendritic cell expansion, proliferation, and functional enhancement. *Exp Hematol.* (2010) 38:877–84. doi: 10.1016/j.exphem.2010.06.004
7. Schwarzenberger P, Huang W, Ye P, Oliver P, Manuel M, Zhang Z, et al. Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. *J Immunol.* (2000) 164:4783–9. doi: 10.4049/jimmunol.164.9.4783
8. Friedman AD. Transcriptional control of granulocyte and monocyte development. *Oncogene.* (2007) 26:6816–28. doi: 10.1038/sj.onc.1210764
9. Strauss L, Sangaletti S, Consonni FM, Szebeni G, Morlacchi S, Totaro MG, et al. RORC1 regulates tumor-promoting "emergency" granulo-monocytopoiesis. *Cancer Cell.* (2015) 28:253–69. doi: 10.1016/j.ccell.2015.07.006
10. Medina E, Hartl D. Myeloid-derived suppressor cells in infection: a general overview. *J Innate Immun.* (2018) 10:407–13. doi: 10.1159/000489830
11. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
12. Sica A, Massarotti M. Myeloid suppressor cells in cancer and autoimmunity. *J Autoimmun.* (2017) 85:117–25. doi: 10.1016/j.jaut.2017.07.010
13. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* (2012) 12:253–68. doi: 10.1038/nri3175
14. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. *Front Immunol.* (2018) 9:1310. doi: 10.3389/fimmu.2018.01310
15. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a mechanism of CD8+T cell tolerance in cancer. *Nat Med.* (2007) 13:828–35. doi: 10.1038/nm1609
16. Yu J, Du W, Yan F, Wang Y, Li H, Cao S, et al. Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *J Immunol.* (2013) 190:3783–97. doi: 10.4049/jimmunol.1201449
17. Shi M, Shi G, Tang J, Kong D, Bao Y, Xiao B, et al. Myeloid-derived suppressor cell function is diminished in aspirin-triggered allergic airway hyperresponsiveness in mice. *J Allergy Clin Immunol.* (2014) 134:1163–74.e16. doi: 10.1016/j.jaci.2014.04.035
18. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med.* (2014) 211:781–90. doi: 10.1084/jem.20131916
19. Pan PY, Ma G, Weber KJ, Ozao-Choy J, Wang G, Yin B, et al. Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res.* (2010) 70:99–108. doi: 10.1158/0008-5472.CAN-09-1882
20. Umemura N, Saio M, Suwa T, Kitoh Y, Bai J, Nonaka K, et al. Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. *J Leukoc Biol.* (2008) 83:1136–44. doi: 10.1189/jlb.0907611

21. Ben-Meir K, Twaik N, Baniyash M. Plasticity and biological diversity of myeloid derived suppressor cells. *Curr Opin Immunol.* (2018) 51:154–61. doi: 10.1016/j.coi.2018.03.015
22. Ai L, Mu S, Wang Y, Wang H, Cai L, Li W, et al. Prognostic role of myeloid-derived suppressor cells in cancers : a systematic review and meta-analysis. *BMC Cancer.* (2018) 18:1–9. doi: 10.1186/s12885-018-5086-y
23. Porta C, Sica A, Riboldi E. Tumor-associated myeloid cells: new understandings on their metabolic regulation and their influence in cancer immunotherapy. *FEBS J.* (2018) 285:717–33. doi: 10.1111/febs.14288
24. Peñaloza HF, Alvarez D, Muñoz-Durango N, Schultz BM, González PA, Kalergis AM, et al. The role of myeloid-derived suppressor cells in chronic infectious diseases and the current methodology available for their study. *J Leukoc Biol.* (2018) 2018:1–16. doi: 10.1002/JLB.MR0618-233R
25. Dorhoi A, Du Plessis N. Monocytic myeloid-derived suppressor cells in chronic infections. *Front Immunol.* (2018) 8:1895. doi: 10.3389/fimmu.2017.01895
26. Tcyganov E, Mastio J, Chen E, Gabrilovich DI. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol.* (2018) 51:76–82. doi: 10.1016/j.coi.2018.03.009
27. Gmünder H, Eck H, Dröge W. Low membrane transport activity for cystine in resting and mitogenically stimulated human lymphocyte preparations and human T cell clones. *Eur J Biochem.* (1991) 201:113–7. doi: 10.1111/j.1432-1033.1991.tb16263.x
28. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res.* (2010) 70:68–77. doi: 10.1158/0008-5472.CAN-09-2587
29. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol.* (2010) 185:3190–8. doi: 10.4049/jimmunol.0903670
30. Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci.* (2010) 107:19961–6. doi: 10.1073/pnas.1014465107
31. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity.* (2005) 22:633–42. doi: 10.1016/j.immuni.2005.03.013
32. Prendergast GC, Malachowski WJ, Mondal A, Scherle P, Muller AJ. Indoleamine 2,3-dioxygenase and its therapeutic inhibition in cancer. *Int Rev Cell Mol Biol.* (2018) 336:175–203. doi: 10.1016/bs.ircmb.2017.07.004
33. Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest.* (2007) 117:1155–66. doi: 10.1172/JCI31422
34. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* (2009) 9:162–74. doi: 10.1038/nri2506
35. Hein FJ, Syvitski JPM. Sea floor gouges and pits in deep fjords, Baffin Island: possible mammalian feeding traces. *Geo-Marine Lett.* (1989) 9:91–4. doi: 10.1002/eji.201747355
36. Thomas AC, Mattila JT. "Of mice and men": Arginine metabolism in macrophages. *Front Immunol.* (2014) 5:479. doi: 10.3389/fimmu.2014.00479
37. Califano JA, Khan Z, Noonan KA, Rudraraju L, Zhang Z, Wang H, et al. Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:30–8. doi: 10.1158/1078-0432.CCR-14-1716
38. Weed DT, Vella JL, Reis IM, De La Fuente AC, Gomez C, Sargi Z, et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory t cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:39–48. doi: 10.1158/1078-0432.CCR-14-1711
39. Hassel JC, Jiang H, Bender C, Winkler J, Sevko A, Shevchenko I, et al. Tadalafil has biologic activity in human melanoma. results of a pilot trial with Tadalafil in patients with metastatic Melanoma (TaMe). *Oncoimmunology.* (2017) 6:e1326440. doi: 10.1080/2162402X.2017.1326440
40. Cantó C, Menzies KJ, Auwerx J. NAD+Metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab.* (2015) 22:31–53. doi: 10.1016/j.cmet.2015.05.023
41. Camacho-Pereira J, Tarragó MG, Chini CCS, Nin V, Escande C, Warner GM, et al. CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. *Cell Metab.* (2016) 23:1127–39. doi: 10.1016/j.cmet.2016.05.006
42. Lee HC. Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. *J Biol Chem.* (2012) 287:31633–40. doi: 10.1074/jbc.R112.349464
43. Karakasheva TA, Waldron TJ, Eruslanov E, Kim SB, Lee JS, O'Brien S, et al. CD38-expressing myeloid-derived suppressor cells promote tumor growth in a murine model of esophageal cancer. *Cancer Res.* (2015) 75:4074–85. doi: 10.1158/0008-5472.CAN-14-3639
44. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood.* (2016) 128:384–94. doi: 10.1182/blood-2015-12-687749
45. Chiarugi A, Dölle C, Felici R, Ziegler M. The NAD metabolome–a key determinant of cancer cell biology. *Nat Rev Cancer.* (2012) 12:741–52. doi: 10.1038/nrc3340
46. Liu G, Bi Y, Shen B, Yang H, Zhang Y, Wang X, et al. SIRT1 limits the function and fate of myeloid-derived suppressor cells in tumors by orchestrating HIF-1α-dependent glycolysis. *Cancer Res.* (2014) 74:727–37. doi: 10.1158/0008-5472.CAN-13-2584
47. Al-Khami AA, Zheng L, Del Valle L, Hossain F, Wyczechowska D, Zabaleta J, et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. *Oncoimmunology.* (2017) 6:e1344804. doi: 10.1080/2162402X.2017.1344804
48. Al-Khami AA, Hossain F, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *J Immunother Cancer.* (2015) 3:O18. doi: 10.1186/2051-1426-3-S2-O18
49. Lu C, Redd PS, Lee JR, Savage N, Liu K. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. *Oncoimmunology.* (2016) 5:e1247135. doi: 10.1080/2162402X.2016.1247135
50. Iwata T, Kondo Y, Kimura O, Morosawa T, Fujisaka Y, Umetsu T, et al. PD-L1+MDSCs are increased in HCC patients and induced by soluble factor in the tumor microenvironment. *Sci Rep.* (2016) 6:39296. doi: 10.1038/srep39296
51. Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE $_2$ pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proc Natl Acad Sci.* (2017) 114:1117–22. doi: 10.1073/pnas.1612920114
52. Zelenay S, Van Der Veen AG, Böttcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell.* (2015) 162:1257–70. doi: 10.1016/j.cell.2015.08.015
53. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J Exp Med.* (2005) 202:931–9. doi: 10.1084/jem.20050715
54. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol.* (2009) 182:4499–506. doi: 10.4049/jimmunol.0802740
55. Condamine T, Gabrilovich DI. Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol.* (2011) 32:19–25. doi: 10.1016/j.it.2010.10.002
56. Rodríguez-Ubreva J, Català-Moll F, Obermajer N, Álvarez-Errico D, Ramirez RN, Company C, et al. Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human myeloid-derived suppressor cells. *Cell Rep.* (2017) 21:154–67. doi: 10.1016/j.celrep.2017.09.018
57. Yan G, Zhao H, Zhang Q, Zhou Y, Wu L, Lei J, et al. A RIPK3-PGE2 circuit mediates myeloid-derived suppressor cell-potentiated colorectal carcinogenesis. *Cancer Res.* (2018) 78:5586–99. doi: 10.1158/0008-5472.CAN-17-3962
58. Sade-Feldman M, Kanterman J, Klieger Y, Ish-Shalom E, Olga M, Saragovi A, et al. Clinical significance of circulating CD33+ CD11bHLA-DR myeloid

cells in patients with stage IV melanoma treated with ipilimumab. *Clin Cancer Res.* (2016) 22:5661–72. doi: 10.1158/1078-0432.CCR-15-3104
59. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, Tarnanidis K, et al. Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. *Clin Cancer Res.* (2015) 21:5453–9. doi: 10.1158/1078-0432.CCR-15-0676
60. Orillion A, Hashimoto A, Damayanti N, Shen L, Adelaiye-Ogala R, Arisa S, et al. Entinostat neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of lung and renal cell carcinoma. *Clin Cancer Res.* (2017) 23:5187–201. doi: 10.1158/1078-0432.CCR-17-0741
61. Suraweera A, O'Byrne KJ, Richard DJ. Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. *Front Oncol.* (2018) 8:92. doi: 10.3389/fonc.2018.00092
62. Linnemann C, Schildberg FA, Schurich A, Diehl L, Hegenbarth SI, Endl E, et al. Adenosine regulates CD8 T-cell priming by inhibition of membrane-proximal T-cell receptor signalling. *Immunology.* (2009) 128:e728–37. doi: 10.1111/j.1365-2567.2009.03075.x
63. Hoskin DW, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review). *Int J Oncol.* (2008) 32:527–35. doi: 10.3892/ijo.32.3.527
64. Ghiringhelli F, Bruchard M, Chalmin F, Rébé C. Production of adenosine by ectonucleotidases: a key factor in tumor immunoescape. *J Biomed Biotechnol.* (2012) 2012:473712. doi: 10.1155/2012/473712
65. Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res.* (2006) 66:1123–31. doi: 10.1158/0008-5472.CAN-05-1299
66. Sica A, Porta C, Morlacchi S, Banfi S, Strauss L, Rimoldi M, et al. Origin and functions of tumor-associated myeloid cells (TAMCs). *Cancer Microenviron.* (2012) 5:133–49. doi: 10.1007/s12307-011-0091-6
67. Gonzalez-Junca A, Driscoll K, Pellicciotta I, Du S, Lo CH, Roy R, et al. Autocrine TGFβ is a survival factor for monocytes and drives immunosuppressive lineage commitment. *Cancer Immunol Res.* (2018) 7:canimm.0310.2018. doi: 10.1158/2326-6066.CIR-18-0310
68. Lee CR, Lee W, Cho SK, Park SG. Characterization of multiple cytokine combinations and TGF-β on differentiation and functions of myeloid-derived suppressor cells. *Int J Mol Sci.* (2018) 19:869. doi: 10.3390/ijms19030869
69. Ryzhov SV, Pickup MW, Chytil A, Gorska AE, Zhang Q, Owens P, et al. Role of TGF-β signaling in generation of $CD39^+$ $CD73^+$ myeloid cells in tumors. *J Immunol.* (2014) 193:3155–64. doi: 10.4049/jimmunol.1400578
70. Li J, Wang L, Chen X, Li L, Li Y, Ping Y, et al. CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-β-mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncoimmunology.* (2017) 6:e1320011. doi: 10.1080/2162402X.2017.1320011
71. Li L, Wang L, Li J, Fan Z, Yang L, Zhang Z, et al. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* (2018) 78:1779–91. doi: 10.1158/0008-5472.CAN-17-2460
72. Wang Y, Tian J, Tang X, Rui K, Tian X, Ma J, et al. Exosomes released by granulocytic myeloid-derived suppressor cells attenuate DSS-induced colitis in mice. *Oncotarget.* (2016) 7:15356–68. doi: 10.18632/oncotarget.7324
73. Geis-Asteggiante L, Dhabaria A, Edwards N, Ostrand-Rosenberg S, Fenselau C. Top-down analysis of low mass proteins in exosomes shed by murine myeloid-derived suppressor cells. *Int J Mass Spectrom.* (2015) 378:264–9. doi: 10.1016/j.ijms.2014.08.035
74. Deng Z, Rong Y, Teng Y, Zhuang X, Samykutty A, Mu J, et al. Exosomes miR-126a released from MDSC induced by DOX treatment promotes lung metastasis. *Oncogene.* (2017) 36:639–51. doi: 10.1038/onc.2016.229
75. Huber V, Vallacchi V, Fleming V, Hu X, Cova A, Dugo M, et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. *J Clin Invest.* (2018) 128:5505–16. doi: 10.1172/JCI98060
76. Liu Y, Wei J, Guo G, Zhou J. Norepinephrine-induced myeloid-derived suppressor cells block T-cell responses via generation of reactive oxygen species. *Immunopharmacol Immunotoxicol.* (2015) 37:359–65. doi: 10.3109/08923973.2015.1059442
77. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol.* (2009) 182:5693–701. doi: 10.4049/jimmunol.0900092
78. Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z, et al. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J Immunol.* (2008) 181:346–53. doi: 10.4049/jimmunol.181.1.346
79. Youn J-I, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J Leukoc Biol.* (2012) 91:167–81. doi: 10.1189/jlb.0311177
80. Beury DW, Carter KA, Nelson C, Sinha P, Hanson E, Nyandjo M, et al. Myeloid-derived suppressor cell survival and function are regulated by the transcription factor Nrf2. *J Immunol.* (2016) 196:3470–8. doi: 10.4049/jimmunol.1501785
81. Jian SL, Chen WW, Su YC, Su YW, Chuang TH, Hsu SC, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. *Cell Death Dis.* (2017) 8:e2779. doi: 10.1038/cddis.2017.192
82. Ohl K, Tenbrock K. Reactive oxygen species as regulators of MDSC-mediated immune suppression. *Front Immunol.* (2018) 9:2499. doi: 10.3389/fimmu.2018.02499
83. Ku AW, Muhitch JB, Powers CA, Diehl M, Kim M, Fisher DT, et al. Tumor-induced MDSC act via remote control to inhibit L-selectin-dependent adaptive immunity in lymph nodes. *Elife.* (2016) 5:e17375. doi: 10.7554/eLife.17375.001
84. Hanson EM, Clements VK, Sinha P, Ilkovitch D, Ostrand-Rosenberg S. Myeloid-derived suppressor cells down-regulate l-selectin expression on CD4+ and CD8+ T cells. *J Immunol.* (2009) 183:937–44. doi: 10.4049/jimmunol.0804253
85. Ostrand-Rosenberg S, Sinha P, Figley C, Long R, Park D, Carter D, et al. Frontline science: myeloid-derived suppressor cells (MDSCs) facilitate maternal–fetal tolerance in mice. *J Leukoc Biol.* (2017) 101:1091–101. doi: 10.1189/jlb.1HI1016-306RR
86. Zhu M, Huang X, Yi S, Sun H, Zhou J. High granulocytic myeloid-derived suppressor cell levels in the peripheral blood predict a better IVF treatment outcome. *J Matern Neonatal Med.* (2017)27:1–6. doi: 10.1080/14767058.2017.1400002
87. Schouppe E, Mommer C, Movahedi K, Laoui D, Morias Y, Gysemans C, et al. Tumor-induced myeloid-derived suppressor cell subsets exert either inhibitory or stimulatory effects on distinct CD8+ T-cell activation events. *Eur J Immunol.* (2013) 43:2930–42. doi: 10.1002/eji.201343349
88. Singh CP, Kumar P, Gupta RK. Generation of solid waste and its management in Govt. Opium and Alkaloids Works, Ghazipur, India. *J Ind Pollut Control.* (1999) 15:51–5. doi: 10.4049/jimmunol.1201018
89. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. Definition of human autoimmunity–autoantibodies versus autoimmune disease. *Autoimmun Rev.* (2010) 9:A259–66. doi: 10.1016/j.autrev.2009.12.002
90. Cripps JG, Gorham JD. MDSC in autoimmunity. *Int Immunopharmacol.* (2011) 11:789–93. doi: 10.1016/j.intimp.2011.01.026
91. Crook KR. Role of myeloid-derived suppressor cells in autoimmune disease. *World J Immunol.* (2014) 4:26. doi: 10.5411/wji.v4.i1.26
92. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Immunol.* (1998) 161:3912–8.
93. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature.* (2010) 464:1293–300. doi: 10.1038/nature08933
94. Lafferty K, Bradley B, Bergman B, Portas M, Haskins K. Pancreatic islet-specific T-cell clones from nonobese diabetic mice. *Proc Natl Acad Sci.* (2006) 86:8000–4. doi: 10.1073/pnas.86.20.8000
95. Wong FS, Visintin I, Wen L, Flavell RA, Janeway CA. CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes

in NOD mice in the absence of CD4 cells. *J Exp Med.* (1996) 183:67–76. doi: 10.1084/jem.183.1.67
96. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes.* (1965) 14:619–33. doi: 10.2337/diab.14.10.619
97. Yin B, Ma G, Yen C-Y, Zhou Z, Wang GX, Divino CM, et al. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *J Immunol.* (2010) 185:5828–34. doi: 10.4049/jimmunol.0903636
98. Hu C, Du W, Zhang X, Wong FS, Wen L. The role of Gr1+ cells after anti-CD20 treatment in type 1 diabetes in nonobese diabetic mice. *J Immunol.* (2012) 188:294–301. doi: 10.4049/jimmunol.1101590
99. Gao X, Liu H, He B, Fu Z. Resistance to streptozotocin-induced autoimmune diabetes in absence of complement C3: myeloid-derived suppressor cells play a role. *PLoS ONE.* (2013) 8:e66334. doi: 10.1371/journal.pone.0066334
100. Whitfield-Larry F, Felton J, Buse J, Su MA. Myeloid-derived suppressor cells are increased in frequency but not maximally suppressive in peripheral blood of type 1 diabetes mellitus patients. *Clin Immunol.* (2014) 153:156–64. doi: 10.1016/j.clim.2014.04.006
101. Carlos D, Costa FRC, Pereira CA, Rocha FA, Yaochite JNU, Oliveira GG, et al. Mitochondrial DNA activates the NLRP3 inflammasome and predisposes to type 1 diabetes in murine model. *Front Immunol.* (2017) 8:164. doi: 10.3389/fimmu.2017.00164
102. Nafee T, Watanabe R, Fregni F. Multiple sclerosis. *Clin Trials Neurol.* (2018) 138:263–95. doi: 10.1007/978-1-4939-7880-9_8
103. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol.* (2011) 164:1079–106. doi: 10.1111/j.1476-5381.2011.01302.x
104. Gaupp S, Pitt D, Kuziel WA, Cannella B, Raine CS. Experimental autoimmune encephalomyelitis (EAE) in CCR2$^{-/-}$mice: susceptibility in multiple strains. *Am J Pathol.* (2003) 162:139–50. doi: 10.1016/S0002-9440(10)63805-9
105. Zhu B, Bando Y, Xiao S, Yang K, Anderson AC, Kuchroo VK, et al. CD11b $^{+}$ Ly-6C ^{hi} Suppressive monocytes in experimental autoimmune encephalomyelitis. *J Immunol.* (2007) 179:5228–37. doi: 10.4049/jimmunol.179.8.5228
106. Mildner A, MacK M, Schmidt H, Brück W, Djukic M, Zabel MD, et al. CCR2+Ly-6Chimonocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain.* (2009) 132:2487–500. doi: 10.1093/brain/awp144
107. King IL, Dickendesher TL, Segal BM. Circulating Ly-6C + myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood.* (2009) 113:3190–7. doi: 10.1182/blood-2008-07-168575
108. Moliné-Velázquez V, Cuervo H, Vila-Del Sol V, Ortega MC, Clemente D, De Castro F. Myeloid-derived suppressor cells limit the inflammation by promoting T lymphocyte apoptosis in the spinal cord of a murine model of multiple sclerosis. *Brain Pathol.* (2011) 21:678–91. doi: 10.1111/j.1750-3639.2011.00495.x
109. Ioannou M, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, et al. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *J Immunol.* (2012) 188:1136–46. doi: 10.4049/jimmunol.1101816
110. Knier B, Hiltensperger M, Sie C, Aly L, Lepennetier G, Engleitner T, et al. Myeloid-derived suppressor cells control B cell accumulation in the central nervous system during autoimmunity. *Nat Immunol.* (2018) 19:1341–51. doi: 10.1038/s41590-018-0237-5
111. Cantoni C, Cignarella F, Ghezzi L, Mikesell B, Bollman B, Berrien-Elliott MM, et al. Mir-223 regulates the number and function of myeloid-derived suppressor cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Acta Neuropathol.* (2017) 133:61–77. doi: 10.1007/s00401-016-1621-6
112. Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature.* (2008) 451:1125–9. doi: 10.1038/nature06607
113. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet.* (2017) 389:2328–37. doi: 10.1016/S0140-6736(17)31472-1
114. Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat Rev Rheumatol.* (2018) 15:9–17. doi: 10.1038/s41584-018-0109-2
115. Dong L, Wang X, Tan J, Li H, Qian W, Chen J, et al. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and treg cells in patients with rheumatoid arthritis. *J Cell Mol Med.* (2014) 18:2213–24. doi: 10.1111/jcmm.12353
116. Jiao Z, Hua S, Wang W, Wang HH, Gao J, Wang X. Increased circulating myeloid-derived suppressor cells correlated negatively with Th17 cells in patients with rheumatoid arthritis. *Scand J Rheumatol.* (2013) 42:85–90. doi: 10.3109/03009742.2012.716450
117. Liu X, Ji B, Sun M, Wu W, Huang L, Sun A, et al. Cell-penetrable mouse forkhead box protein 3 alleviates experimental arthritis in mice by up-regulating regulatory T cells. *Clin Exp Immunol.* (2015) 181:87–99. doi: 10.1111/cei.12630
118. Cribbs AP, Kennedy A, Penn H, Amjadi P, Green P, Read JE, et al. Methotrexate restores regulatory T cell function through demethylation of the FoxP3 upstream enhancer in patients with rheumatoid arthritis. *Arthritis Rheumatol.* (2015) 67:1182–92. doi: 10.1002/art.39031
119. Li M, Zhu D, Wang T, Xia X, Tian JWS. Roles of myeloid-derived suppressor cell subpopulations in autoimmune arthritis. *Front Immunol.* (2018) 9:2849. doi: 10.3389/fimmu.2018.02849
120. Fujii W, Ashihara E, Hirai H, Nagahara H, Kajitani N, Fujioka K, et al. Myeloid-derived suppressor cells play crucial roles in the regulation of mouse collagen-induced arthritis. *J Immunol.* (2013) 191:1073–81. doi: 10.4049/jimmunol.1203535
121. Egelston C, Kurkó J, Besenyei T, Tryniszewska B, Rauch TA, Glant TT, et al. Suppression of dendritic cell maturation and T cell proliferation by synovial fluid myeloid cells from mice with autoimmune arthritis. *Arthritis Rheum.* (2012) 64:3179–88. doi: 10.1002/art.34494
122. Park MJ, Lee SH, Kim EK, Lee EJ, Baek JA, Park SH, et al. Interleukin-10 produced by myeloid-derived suppressor cells is critical for the induction of Tregs and attenuation of rheumatoid inflammation in mice. *Sci Rep.* (2018) 8:3753. doi: 10.1038/s41598-018-21856-2
123. Guo C, Hu F, Yi H, Feng Z, Li C, Shi L, et al. Myeloid-derived suppressor cells have a proinflammatory role in the pathogenesis of autoimmune arthritis. *Ann Rheum Dis.* (2016) 75:278–85. doi: 10.1136/annrheumdis-2014-205508
124. Zhang H, Wang S, Huang Y, Wang H, Zhao J, Gaskin F, et al. Myeloid-derived suppressor cells are proinflammatory and regulate collagen-induced arthritis through manipulating Th17 cell differentiation. *Clin Immunol.* (2015) 157:175–86. doi: 10.1016/j.clim.2015.02.001
125. Zhang H, Huang Y, Wang S, Fu R, Guo C, Wang H, et al. Myeloid-derived suppressor cells contribute to bone erosion in collagen-induced arthritis by differentiating to osteoclasts. *J Autoimmun.* (2015) 65:82–9. doi: 10.1016/j.jaut.2015.08.010
126. Castrejon I, Nika A, Sequeira W, Jolly M. Systemic lupus erythematosus. *Comorbidity Rheum Dis.* (2017) 6:145–63. doi: 10.1007/978-3-319-59963-2_6
127. Lourenço EV, Wong M, Hahn BH, Palma-Diaz MF, Skaggs BJ. Laquinimod delays and suppresses nephritis in lupus-prone mice and affects both myeloid and lymphoid immune cells. *Arthritis Rheumatol.* (2014) 66:674–85. doi: 10.1002/art.38259
128. Li P, Lin W, Zheng X. IL-33 neutralization suppresses lupus disease in lupus-prone mice. *Inflammation.* (2014) 37:824–32. doi: 10.1007/s10753-013-9802-0
129. Thaxton JE, Liu B, Zheng P, Liu Y, Li Z. Deletion of CD24 impairs development of heat shock protein gp96-driven autoimmune disease through expansion of myeloid-derived suppressor cells. *J Immunol.* (2014) 192:5679–86. doi: 10.4049/jimmunol.1302755
130. Wu H, Zhen Y, Ma Z, Li H, Yu J, Xu ZG, et al. Arginase-1-dependent promotion of TH17 differentiation and disease progression by MDSCs in systemic lupus erythematosus. *Sci Transl Med.* (2016) 8:331ra40. doi: 10.1126/scitranslmed.aae0482
131. Zhang D, Xu J, Ren J, Ding L, Shi G, Li D, et al. Myeloid-derived suppressor cells induce podocyte injury through increasing reactive oxygen species in lupus nephritis. *Front Immunol.* (2018) 9:1443. doi: 10.3389/fimmu.2018.01443

132. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature.* (2007) 448:427–34. doi: 10.1038/nature06005
133. Abraham C, Cho JH. IL-23 and Autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. *Annu Rev Med.* (2009) 60:97–110. doi: 10.1146/annurev.med.60.051407.123757
134. Moschen AR. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroenterol Hepatol.* (2018) 16:185–96. doi: 10.1038/s41575-018-0084-8
135. Haile LA, von Wasielewski R, Gamrekelashvili J, Krüger C, Bachmann O, Westendorf AM, et al. Myeloid-derived suppressor cells in inflammatory bowel disease: a new immunoregulatory pathway. *Gastroenterology.* (2008) 135:871–81.e5. doi: 10.1053/j.gastro.2008.06.032
136. Westendorf AM, Fleissner D, Deppenmeier S, Gruber AD, Bruder D, Hansen W, et al. Autoimmune-mediated intestinal inflammation-impact and regulation of antigen-specific CD8+T cells. *Gastroenterology.* (2006) 131:510–24. doi: 10.1053/j.gastro.2006.05.015
137. Zhang J, Wang B, Zhang W, Wei Y, Bian Z, Zhang CY, et al. Protein tyrosine phosphatase 1B deficiency ameliorates murine experimental colitis via the expansion of myeloid-derived suppressor cells. *PLoS ONE.* (2013) 8:e70828. doi: 10.1371/journal.pone.0070828
138. Däbritz J, Judd LM, Chalinor H V., Menheniott TR, Giraud AS. Altered gp130 signalling ameliorates experimental colitis via myeloid cell-specific STAT3 activation and myeloid-derived suppressor cells. *Sci Rep.* (2016) 6:20584. doi: 10.1038/srep20584
139. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation program of Ly6C ^{hi} monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J Exp Med.* (2012) 209:139–55. doi: 10.1084/jem.20101387
140. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity.* (2009) 31:502–12. doi: 10.1016/j.immuni.2009.06.025
141. Ostanin DV, Kurmaeva E, Furr K, Bao R, Hoffman J, Berney S, et al. Acquisition of antigen-presenting functions by neutrophils isolated from mice with chronic colitis. *J Immunol.* (2012) 188:1491–502. doi: 10.4049/jimmunol.1102296
142. Xi Q, Li Y, Dai J, Chen W. High frequency of mononuclear myeloid-derived suppressor cells is associated with exacerbation of inflammatory bowel disease. *Immunol Invest.* (2015) 44:279–87. doi: 10.3109/08820139.2014.999937
143. McIntosh KR, Drachman DB. Induction of apoptosis in activated T cell blasts by suppressive macrophages: a possible immunotherapeutic approach for treatment of autoimmune disease. *Cell Immunol.* (1999) 193:24–35. doi: 10.1006/cimm.1998.1445
144. Cao LY, Chung JS, Teshima T, Feigenbaum L, Cruz PD, Jacobe HT, et al. Myeloid-derived suppressor cells in psoriasis are an expanded population exhibiting diverse T-cell–suppressor mechanisms. *J Invest Dermatol.* (2016) 136:1801–10. doi: 10.1016/j.jid.2016.02.816
145. Ilkovitch D, Ferris LK. Myeloid-derived suppressor cells are elevated in patients with psoriasis and produce various molecules. *Mol Med Rep.* (2016) 14:3935–40. doi: 10.3892/mmr.2016.5685
146. Soler DC, Young AB, Fiessinger L, Galimberti F, Debanne S, Groft S, et al. Increased, but functionally impaired, CD14+ HLA-DR–/low myeloid-derived suppressor cells in psoriasis: a mechanism of dysregulated T cells. *J Invest Dermatol.* (2016) 136:798–808. doi: 10.1016/j.jid.2015.12.036
147. Kerr EC, Raveney BJE, Copland DA, Dick AD, Nicholson LB. Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J Autoimmun.* (2008) 31:354–61. doi: 10.1016/j.jaut.2008.08.006
148. Raveney BJE, Copland DA, Dick AD, Nicholson LB. TNFR1-dependent regulation of myeloid cell function in experimental autoimmune uveoretinitis. *J Immunol.* (2009) 183:2321–9. doi: 10.4049/jimmunol.0901340
149. Hou Y, Feng Q, Xu M, Li GS, Liu XN, Sheng Z, et al. High-dose dexamethasone corrects impaired myeloid-derived suppressor cell function via Ets1 in immune thrombocytopenia. *Blood.* (2016) 127:1587–97. doi: 10.1182/blood-2015-10-674531
150. Rosenblum MD, Gratz IK, Paw JS, Abbas AK. Treating human autoimmunity: Current practice and future prospects. *Sci Transl Med.* (2012) 4:125sr1. doi: 10.1126/scitranslmed.3003504
151. Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science.* (1992) 257:789–92. doi: 10.1126/science.1323143
152. Maxwell LJ, Singh JA. Abatacept for rheumatoid arthritis: a cochrane systematic review. *J Rheumatol.* (2010) 37:234–45. doi: 10.3899/jrheum.091066
153. Mease P, Genovese MC, Gladstein G, Kivitz AJ, Ritchlin C, Tak PP, et al. Abatacept in the treatment of patients with psoriatic arthritis: Results of a six-month, multicenter, randomized, double-blind, placebo-controlled, phase II trial. *Arthritis Rheum.* (2011) 63:939–48. doi: 10.1002/art.30176
154. Schatz D, Monzavi R, Marks JB, Becker DJ, Raskin P, Skyler JS, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet.* (2011) 378:412–9. doi: 10.1016/s0140-6736(11)60886-6
155. Lai Y, Dong C. Therapeutic antibodies that target inflammatory cytokines in autoimmune diseases. *Int Immunol.* (2016) 28:181–8. doi: 10.1093/intimm/dxv063
156. Kurkó J, Vida A, Ocskó T, Tryniszewska B, Rauch TA, Glant TT, et al. Suppression of proteoglycan-Induced autoimmune arthritis by myeloid-Derived suppressor cells generated *in vitro* from murine bone marrow. *PLoS ONE.* (2014) 9:e111815. doi: 10.1371/journal.pone.0111815
157. Nishimura K, Saegusa J, Matsuki F, Akashi K, Kageyama G, Morinobu A. Tofacitinib facilitates the expansion of myeloid-derived suppressor cells and ameliorates arthritis in SKG mice. *Arthritis Rheumatol.* (2015) 67:893–902. doi: 10.1002/art.39007
158. Park MJ, Lee SH, Kim EK, Lee EJ, Park SH, Kwok SK, et al. Myeloid-derived suppressor cells induce the expansion of regulatory b cells and ameliorate autoimmunity in the sanroque mouse model of systemic lupus erythematosus. *Arthritis Rheumatol.* (2016) 68:2717–27. doi: 10.1002/art.39767
159. Ma Y, Bernstein CN, Guan Q, Qing G, Lu L, Moreno S, et al. The role and potential therapeutic application of myeloid-derived suppressor cells in TNBS-induced colitis. *J Leukoc Biol.* (2013) 94:803–11. doi: 10.1189/jlb.0113050
160. Su H, Cong X, Liu YL. Transplantation of granulocytic myeloid-derived suppressor cells (G-MDSCs) could reduce colitis in experimental murine models. *J Dig Dis.* (2013) 14:251–8. doi: 10.1111/1751-2980.12029
161. Hsieh CC, Lin CL, He JT, Chiang M, Wang Y, Tsai YC, et al. Administration of cytokine-induced myeloid-derived suppressor cells ameliorates renal fibrosis in diabetic mice. *Stem Cell Res Ther.* (2018) 9:183. doi: 10.1186/s13287-018-0915-0
162. Greening DW, Gopal SK, Xu R, Simpson RJ, Chen W. Exosomes and their roles in immune regulation and cancer. *Semin Cell Dev Biol.* (2015) 40:72–81. doi: 10.1016/j.semcdb.2015.02.009
163. Zöller M, Zhao K, Kutlu N, Bauer N, Provaznik J, Hackert T, et al. Immunoregulatory effects of myeloid-derived suppressor cell exosomes in mouse model of autoimmune Alopecia Areata. *Front Immunol.* (2018) 9:1279. doi: 10.3389/fimmu.2018.01279
164. Munn DH, Serody JS, Ochoa AC, Panoskaltsis-Mortari A, Veenstra R, Blazar BR, et al. Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13. *Blood.* (2010) 116:5738–47. doi: 10.1182/blood-2010-06-287839
165. Wang L, Lu LL, Wagner T, Chou H-S, Charles R, Hsieh C-C, et al. Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated enhancement of T regulatory cells. *Transplantation.* (2011) 93:272–82. doi: 10.1097/tp.0b013e31823ffd39
166. Marigo I, Bosio E, Solito S, Mesa C, Fernandez A, Dolcetti L, et al. Tumor-induced tolerance and immune suppression depend on the C/EBPβ transcription factor. *Immunity.* (2010) 32:790–802. doi: 10.1016/j.immuni.2010.05.010

167. Schmidt K, Zilio S, Schmollinger JC, Bronte V, Blankenstein T, Willimsky G. Differently immunogenic cancers in mice induce immature myeloid cells that suppress CTL *in vitro* but not *in vivo* following transfer. *Blood.* (2013) 121:1740–8. doi: 10.1182/blood-2012-06-436568
168. Sade-Feldman M, Kanterman J, Ish-Shalom E, Elnekave M, Horwitz E, Baniyash M. Tumor necrosis factor-α blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. *Immunity.* (2013) 38:541–54. doi: 10.1016/j.immuni.2013.02.007
169. Greifenberg V, Ribechini E, Rößner S, Lutz MB. Myeloid-derived suppressor cell activation by combined LPS and IFN-γ treatment impairs DC development. *Eur J Immunol.* (2009) 39:2865–76. doi: 10.1002/eji.200939486

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REVIEW
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Myeloid-Derived Suppressor Cells: Not Only in Tumor Immunity

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Since the realization that immature myeloid cells are powerful modulators of the immune response, many studies on "myeloid-derived suppressor cells" (MDSCs) have documented their ability to promote tumor progression in melanoma and other cancers. Whether MDSCs are induced solely pathologically in tumorigenesis, or whether they also represent physiological immune control mechanisms, is not well-understood, but is particularly important in the light of ongoing attempts to block their activities in order to enhance anti-tumor immunity. Here, we briefly review studies which explore (1) how best to identify MDSCs in the context of cancer and how this compares to other conditions in humans; (2) what the suppressive mechanisms of MDSCs are and how to target them pharmacologically; (3) whether levels of MDSCs with various phenotypes are informative for clinical outcome not only in cancer but also other diseases, and (4) whether MDSCs are only found under pathological conditions or whether they also represent a physiological regulatory mechanism for the feedback control of immunity. Studies unequivocally document that MDSCs strongly influence cancer outcomes, but are less informative regarding their relevance to infection, autoimmunity, transplantation and aging, especially in humans. So far, the results of clinical interventions to reverse their negative effects in cancer have been disappointing; thus, developing differential approaches to modulate MSDCs in cancer and other diseases without unduly comprising any normal physiological function requires further exploration.

Keywords: MDSC, cancer immunity, obesity, autoimmunity, aging, transplantation, infectious disease

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INTRODUCTION

Myeloid cells encompassing monocytes, macrophages, dendritic cells, polymorphonuclear granulocytic cells and others are continuously generated from hematopoietic stem cells through multi-step differentiation processes. The presence of cancer can skew hematopoiesis toward myelopoiesis, probably mediated by pro-inflammatory factors (1). Interestingly, similar phenomena occur in overtly cancer-free aging, presumably for the same reasons (2). Immature myeloid cells at certain stages of differentiation may act in an immunosuppressive manner and are therefore designated "myeloid-derived suppressor cells" (MDSCs). MDSCs have been most intensively studied in the context of cancer (3). They are a very heterogeneous group of mononuclear and polymorphonuclear myeloid cells, normally present at very low numbers in healthy individuals, but may accumulate under disease conditions (4)—or potentially during natural aging (5, 6) or with psychological stress (7). These influences, in addition to heterogeneity in the differentiation trajectory for myeloid lineage cells, means that there are no validated unique

phenotypic markers for MDSCs and they can only be unequivocally identified using functional assays (8). Unfortunately, these biological assays have many drawbacks in terms of reproducibility, standardization, requirements for large amounts of cells, etc. Therefore, despite the lack of robust associations between phenotypes and function, many studies rely on the former as a surrogate for the latter. The field is further complicated by inherent differences between humans and animal models, mostly mice, which make preclinical studies challenging (9, 10). Hence, in this article, we will attempt to briefly review (1) how best to identify MDSCs in humans; (2) what are their suppressive mechanisms and how to target them pharmacologically; (3) if their levels of MDSCs informative for clinical outcomes; and (4) whether MDSCs are only important in pathology or whether they also represent physiological regulatory mechanisms for the feedback control of immunity. In this latter section, relying mostly on studies in mouse models which can be experimentally manipulated, a basis is established for translating animal data to areas less well-documented than cancer in humans. Because the role of MDSCs in cancer has been subject to considerable recent scrutiny [reviewed in (11, 12)], we will only very briefly cover this issue and focus more on the importance of MDSCs in other conditions.

IDENTIFICATION OF MDSCS

Characteristics of MDSCs

MDSCs were first phenotypically identified in tumor-bearing mice by their expression of CD11b and Gr-1 (13, 14). This is not possible in humans because there is no Gr-1 homolog. As a simplification, in humans, functional MDSCs are generally recognized to be either mononuclear and monocytic (M-MDSCs, expressing surface CD14), or polymorphonuclear and granulocytic (PMN-MDSCs, expressing CD15) (8). Accurate phenotypic characterization requiring functional studies is challenging in humans, not least because healthy people possess very few MDSCs and accessing sufficient amounts of blood from patients, especially those with cancer, is not trivial. For multi-center studies there is the additional limitation that the requirement for cryopreservation of samples means that only M-MDSCs can be readily analyzed because PMN-MDSC do not readily survive freezing (15). Furthermore, as the source of this material is mostly peripheral blood, the method of cell isolation, freezing and storage adds further variation. Perhaps because of this, and the lack of the human CD11b-Gr-1 murine standard, it is commonly the experience that individual laboratories are using very different approaches to phenotyping and functional testing. In the context of applying MDSC data for use as biomarkers for clinical prognosis and prediction, this raises concerns about routine applicability of any MDSC methodology. Many efforts have been addressed toward attempting to resolve this issue. Recommendations based on multi-center collaborative studies are beginning to make some inroads into solving this problem, for example, by paralleling subtypes of MDSCs defined phenotypically with their suppressive activity in functional assays (8). However, the problem is compounded by the fact that sorting MDSCs for use in functional tests is itself problematic and while several different approaches remain in use, a great deal of heterogeneity persists in the literature. Separating cells by magnetic bead sorting is limited by the small number of markers that can be used but multi-parametric fluorescence-activated cell sorting (FACS) is expensive, slow and can alter cell characteristics. It seems safe to conclude that there is no optimal approach to isolate MDSCs and attempts to increase the sophistication of phenotyping and biological assays of suppression will remain challenging. Knowledge of the mechanisms employed by MDSCs to suppress immune responses may allow biochemical and/or genetic analyses to circumvent cumbersome phenotyping and functional assays in the future (see following sections).

Efforts to better characterize the surface phenotype of MDSCs have included the use of CyTOF to increase the number of possible subsets detectable. Unfortunately, CyTOF cannot sort viable cells for functional testing, although this may not be necessary when the desire is only to establish biomarkers relevant to cancer patient survival. Few data were available at the time of writing. A pilot study used CyTOF to examine extended phenotypes in 5 melanoma patients (16), and in our own pilot study of 27 stage IV melanoma patients we were unable to identify an extended phenotype that correlated with overall survival better than the basic M-MDSC phenotype $CD14^{+}CD11c^{+}HLA\text{-}DR^{-/lo}$, despite including over 30 markers (17). However, this pilot study still included only very few patients and work is ongoing. Considering that monocytes could themselves be viewed as immature macrophages, it is not surprising that phenotypes close to the classical monocyte phenotype delineate populations of cells with regulatory activities (18). Multi-parametric FACS has also increased in sophistication since its introduction, as illustrated for example in the report of the CIMT multi-center phenotyping harmonization study (19), but these more extended phenotypes may also not prove any better than the simple monocyte phenotype. Thus far, the type of transcriptomics approach so widely used for analyzing T and B cell populations yielded relatively sparse information on potential gene expression patterns in the different populations of MDSCs (20), so progress might be possible at this level in the future. The increasing availability of databases and analytical algorithms to assess the presence of immune cells within tumors encourage the belief that this approach will soon yield valuable insights (21).

Induction of MDSCs

What is clear from both animal and human studies is the strong influence of an inflammatory or anti-inflammatory microenvironment on the differentiation of myeloid precursors into functional cells (11, 14, 22). Much of what we know about the induction of M-MDSCs in humans derives from *in vitro* experiments sequentially culturing monocytes with different cytokines and chemokines to mimic the inflammatory/anti-inflammatory microenvironment, and then analyzing the phenotypes and functions of the derived cells (23). Modifications of these approaches include cultures containing tumor cells to induce MDSCs (24, 25), which also provide an opportunity to investigate how to prevent their induction, as for example in

Janssen et al. (26). These properties can then be compared with cells from patients or healthy controls.

Such monocyte-derived cells can be made to differentiate into immune stimulatory cells (predominantly dendritic cells, DCs) or tolerogenic cells reminiscent of MDSCs, depending on the culture protocol. Many experiments expose monocytes to a mixture of GM-CSF and IL-4 for a few days to generate activated immature cells which can then be caused to mature into DCs by adding inflammatory cytokines. However, if IL-10 is present from the beginning of culture, resulting cells maintain high levels of CD14 but downregulate HLA-DR, a classic MDSC phenotype, and also express characteristic molecules like glucocorticoid-induced-tumor-necrosis-factor-receptor-related-protein (GITR) (27). Thus, at least some of the anti-inflammatory and immunosuppressive effects of IL-10 may be mediated in this manner. It is the balance of soluble factors and other stimuli during the differentiation of the precursors that clearly affects the final outcome, complex to examine *in vitro*, near impossible *in vivo* in humans. Nonetheless, *in vitro* studies can point to targets addressable in patients, for example altering the PGE_2:COX2 ratio (28), as further discussed below.

MEASUREMENT AND MECHANISMS OF SUPPRESSION

Assays and Mechanisms of Suppression

By separating candidate M-MDSCs on the basis of their phenotype and testing each subset for suppressive activity, it was hoped to identify the most biologically relevant phenotype (with the caveat that *in vitro* suppressor assays will still only be biomarkers that need to be associated with a robust clinical outcome in order to be meaningful). M-MDSCs produce multiple molecules that could be candidates for mediating suppression. Of these, the "metabolic inhibitors" arginase-1 (ARG-1) and indolamine-2,3-dioxygenase- (IDO), catabolizing arginine and tryptophan, respectively, have been extensively investigated, but many other molecules such as the cytokines IL-10 and TGF-ß, as well as nitric oxide (NO) are likely to be involved as well (10, 29). Whereas the functional activity of murine MDSCs can be evaluated in antigen-specific assays, the result of human MDSC inhibitory activity is commonly evaluated using crude assays involving pan-T cell stimulation and measuring decreased cell division or cytokine production in the presence of titrated numbers of MDSCs. Again, these biological assays are quite variable and difficult to compare between laboratories (8). One way to try and reduce some variability is for journals to request adherence to standardized methods reporting parameters so that investigators are at least assured that they are using the same approach. Guidelines such as MIATA (minimal information on T cell assays) do exist for this purpose and should be strongly encouraged (30).

Interventions to Alleviate Suppression

It is emerging that certain chemotherapy agents currently approved for clinical application reduce MDSC levels, and may in fact rely on this facet of their function for a large part of their anti-cancer therapeutic activity. On the other hand, some chemotherapeutic drugs may have the opposite effect and enhance MDSC function. For example, in colon cancer regimes 5-FU has an anti-MDSC effect but Irinotecan a pro-MDSC effect (31). Several chemotherapeutic drugs, even at low doses, as well as those affecting the maturation of myeloid cells (e.g., all-trans retinoic acid, ATRA) may prevent MDSC function (32). A very small ongoing trial combining checkpoint blockade with ATRA treatment in stage IV metastatic melanoma is expected to report next year [2020] (see https://ClinicalTrials.gov/show/NCT02 403778) with an interim analysis available now (33). Anti-inflammatory agents also impinge on the induction and maintenance of MDSC function, and several approaches utilizing drugs modulating inflammatory pathways are ongoing [see (34) for a recent review]. It may prove more effective to target the induction of MDSCs than targeting their suppressive mechanisms and products, as notoriously illustrated by the recent failure of a phase III trial to block IDO (35). While there could have been many reasons for this failure, murine studies demonstrating the strong homeostatic compensation for elimination of tumor-infiltrating MDSCs supports the concept that targeting MDSC induction may be the most effective approach (36). Interest remains high in developing means to successfully modulate this pathway. Given the intrinsic redundancy in immunological feedback control mechanisms, it is likely that multiple pathways will need to be modulated to succeed in this aim, as discussed for example in Ostrand-Rosenberg (14). Of the many additional approaches that could be explored, differentiation of the immature MDSCs away from a suppressive phenotype by physiological induction, for example, of IL-12 by innate immune agonists rather than pharmacological agents, may be a fruitful approach (37).

ARE LEVELS OF MDSCS INFORMATIVE FOR CANCER PATIENT SURVIVAL?

The majority of correlative data between MDSCs and clinical outcomes pertains to cancer, where the presence of cells with phenotypes of one type or another have been associated with patient survival in many different tumors including melanoma (38), breast (39), lung (40), and others. These data nearly always refer to assessments on peripheral blood and can only be considered biomarkers for clinical outcome, rather than providing mechanistic inference. However, our studies assessing the ability of patients' PBMCs to respond to candidate tumor-associated antigens *in vitro*, combined with patient MDSC frequency, do show correlations with survival (39, 41). This suggests that suppression of anti-cancer antigen responses due to high levels of MDSCs (but not regulatory T cells) does impinge on clinical outcome. However, changes in levels of MDSCs during checkpoint blockade (with single agent ipilimumab in melanoma) were not associated with overall survival (42). Thus, despite the predictive value of baseline levels of MDSCs, whether or not these changed during treatment with ipilimumab, was not related to responsiveness. We are currently investigating whether the same holds true for the current standard of care that has superceded single agent ipilimumab (anti-PD-1 with or without

ipilimumab). There is also evidence that higher levels of MDSCs also result in poorer responses to cancer vaccines (43).

ARE MDSCS EXCLUSIVELY PATHOLOGICAL?

MDSCs in Infectious Disease

Combating acute infections requires an inflammatory response which may also cause "collateral" tissue damage, normally repaired once the infection is resolved. However, when the response becomes chronic, homeostasis requires a balance between continued immune surveillance and protection against tissue damage resulting from inflammatory mediators. This may be the reason why inflammatory cytokines and chemokines induce and maintain MDSCs as part of the immune feedback control in chronic infection and why MDSC can induce regulatory T cells (44). This would be a physiological requirement, but could also lead to excessive immunosuppression if unbalanced. What is the evidence for this hypothesis? Prime examples include chronic bacterial (e.g., tuberculosis [TB]) and viral (e.g., Hepatitis C virus [HCV]) infections. While most data are derived from mouse models, myeloid cells with many of the features of MDSCs as defined in cancer patients are found in the blood of TB patients, with reduced numbers after treatment, potentially suggesting a pathological effect of these cells when present in large amounts (45). The same may be true for chronic HCV (46) and HBV infection (47); however, a later study found no relationship between MDSCs and HCV infection (48). This raises the question so prevalent in cancer studies as to the identification and standardization of detection techniques for these cells. Nonetheless, the weight of opinion and data in the literature strongly suggest that MDSCs play important roles in chronic infections, and that these are always or nearly always pathological as more clearly discerned from mouse studies (although the latter are often not at all reliable guides to clinical conditions) (49).

Are there any indications that MDSCs might play beneficial roles in disease? This seems not to be the case for M-MDSCs, but there is some evidence for a beneficial role of PMN-MDSCs in resolving acute HBV infection and preventing liver damage [reviewed in Dorhoi et al. (49)]. Thus, it is conceivable that PMN-MDSCs can have protective effects in limiting tissue damage caused by inflammation in acute infections, but M-MDSCs induced in a chronic inflammatory environment are likely always to exert pathogenic influences. This remains a hypothesis to be rigorously tested.

MDSCs in Autoimmunity

The role of MDSCs in autoimmunity is hotly debated and controversial (50). Clearly, the normal inflammatory response in infectious disease is self-limiting, but in chronic autoimmune conditions this regulation is disrupted. One factor contributing to this could be a dearth of MDSCs allowing destructive processes to continue. The question is therefore whether MDSCs do have key roles in promoting or maintaining tolerance and whether the mechanism might be via modulating T cell responses. Here again, there are relatively few data. Although only a pilot study, in rheumatoid arthritis, PMN-MDSCs present in the synovial fluid were suggested to contribute to inhibiting autoreactive T cells (51), but clearly did not prevent pathology. The same is true for multiple sclerosis, where there also appears to be no data on M-MDSCs in humans (52). A more recent paper on the rare disease cryopyrin-associated periodic syndromes (CAPS, caused by NLRP3 mutations and consequent overproduction of IL-1β) suggested that MDSCs might act in an anti-inflammatory manner and exert beneficial effects (53). MDSCs have also been implicated in the differential pathology of asthma and COPD (54). Relative to large amounts of work in mice, which may be translatable to humans only with difficulty, there are vanishingly small amounts of data on the role of MDSCs in human autoimmunity, identifying an important unmet need for understanding this pathological process.

MDSCs in Aging

Considering the strong relationship between MDSCs and cancer, and aging and cancer incidence (55), as well as incidence of other non-communicable age-related diseases, age-related trends pertaining to MDSC expansion and function would be expected. Although, again, little is known about the impact of age on MDSCs in humans, this is indeed the case in mice, which exhibit higher levels of MDSCs in the bone marrow, spleen and peripheral lymph nodes with increasing age (56–58). Importantly for immune function at older age, MDSCs down-regulate L-selectin (CD62L) on naive T cells through their expression of the protease ADAM17 (59). L-selectin on naive T cells is essential for their entry into lymph nodes where they become activated. Aged mice have even lower levels of CD62L on their naive T cells because they have higher levels of MDSCs. Additionally, older mice have a reduced ability to clear MDSCs following experimentally induced expansion (60). Similarly, in humans, compared to young adults (<60 years old), older community-dwelling individuals (61–76 years old) and the frail, institutionalized elderly (67–99 years old) exhibit significantly higher peripheral blood levels of $CD33^{+}$HLA-DR-negative MDSCs, especially the $CD11b^{+}CD15^{+}$ subset (5). Higher frequencies of MDSCs in older humans may be limited to the PMN-MDSCs—one small study on 12 people >80 years of age reported higher levels of PMN-MDSCs but not M-MDSCs (6). Interestingly, the mechanisms involved in age-related increases of MDSCs appear to be at least partly determined by well-known aging-associated processes, namely cellular senescence and inflammation, and possibly the skewing of hematopoiesis away from the lymphoid toward the myeloid lineage. Cellular senescence contributes to age-related functional outcomes and systemic inflammation (i.e., the senescence-associated secretory phenotype, SASP) (61). Using a p27 senescence-inducible system, it was found that senescent fibroblasts, via the secretion of proinflammatory cytokines such as IL-6, promoted the local accumulation of MDSCs (62). This coincides with findings from a study of older adults with idiopathic pulmonary fibrosis, suggesting that MDSCs tend to accumulate near fibrotic lesions (63), which are enriched in senescent fibroblasts (64). The role of inflammation has also been demonstrated in naturally aged and in accelerated aging (i.e.,

ERCC1-deficient) mouse models. Aged and ERCC1-deficient mice exhibited significantly higher frequencies of MDSCs as well as elevated MDSC NF-kB transcriptional activity in the absence of exogenous stimulation (65). Although indirect in humans, there are several lines of evidence connecting MDSCs to the prevalence of age-related diseases, and in some cases, the severity of those diseases. MDSCs have been found to be higher in patients suffering from rheumatoid arthritis and osteoarthritis, especially so for patients with more severe forms of the disease (i.e., elevated CRP and elevated pain levels) (66). This was also observed in patients with amnestic cognitive impairment, where MDSC levels were significantly higher than healthy controls (67). Interestingly, another group found that the frequency of M-MDSCs was significantly higher in the blood of patients with mild forms of Alzheimer's disease (assessed using the clinical dementia rating), but not more severe forms, and that M-MDSCs from these mild cases were more suppressive *ex vivo* (68); the same was true in multiple sclerosis (69). Finally, as mentioned above, MDSCs have been shown to be higher in older adults with idiopathic pulmonary fibrosis (63), as well as incident cases of Parkinson's disease, where they are nearly five-fold above levels in healthy controls (70). Taken together, all these studies strongly suggest a role for aging in the expansion and function of MDSCs, which promote disease in older adults. That being said, further work in humans is vital, particularly the age-related mechanisms that influence MDSCs and the longitudinal relationship between MDSC frequency and age-related disease and other important outcomes.

MDSCs in Obesity

Obesity and high fat diet (HFD) are established risk factors that contribute to increased cancer incidence, increased tumor progression, and increased cancer mortality (71, 72). Obesity is accompanied by multiple biological changes that contribute to malignancy. One such change is the low-grade inflammation associated with adipose tissue due to the production of TNF, IL-1β, IL-6, and prostaglandin E2 (PGE2). These pro-inflammatory mediators are produced by adipocytes as well as by adipose-infiltrating macrophages (73), and directly impact cancer risk and progression, leading to the concept that obesity-associated inflammation is an important mechanism by which obesity facilitates malignancy (74). The chronic low-grade inflammatory milieu present in obese tissue is similar to the pro-inflammatory environment present in many solid tumors that leads to the induction of MDSCs. Lipids themselves also drive the accumulation and suppressive potency of MDSCs. Mouse and human studies have shown that polyunsaturated fatty acids (PUFAs), such as omega-3 fatty acids, and fatty acid metabolism increase the generation and suppressive activity of MDSCs (75, 76). Given the role of chronic inflammation as a driver of MDSCs and the prevalence of lipid in obese individuals, it is not unexpected that M-MDSC are elevated in obese humans (77).

Studies examining the function of MDSCs in obese individuals have to date only been conducted in mice. Two experimental systems have been used to generate overweight/obese mice: (i) Ob/Ob mice are genetically leptin-deficient and therefore lack appetite control and rapidly become overweight. (ii) Inbred mice fed a HFD consisting of 60% fat become overweight/obese relative to mice kept on a low fat diet (LFD) consisting of 10% fat. In both models M-MDSC and PMN-MDSC levels increase with increasing weight gain (78). As expected, overweight mice on a HFD and with elevated levels of MDSCs have more rapidly growing tumors and more extensive metastatic disease, and their T cells are less activated by antigen. Depletion of MDSCs in HFD mice reverts tumor growth rates to that observed in LFD mice and restores antigen-driven T cell activation, while depletion of both MDSCs and $CD4^+$ and $CD8^+$ T cells increases tumor growth rate. MDSCs from HFD mice also are more efficient suppressors of antigen-activated T cells, and tumor-infiltrating MDSCs from HFD mice express elevated levels of PD-L1. The latter effect is most likely the result of higher levels of IFNγ in the tumors of HFD mice (79). Elevated levels of MDSC and PD-L1 on MDSC may also provide a broader target for PD-1 checkpoint blockade immunotherapy and explain why PD-1 therapy is more efficacious in obese cancer patients (80).

Increased levels of MDSCs in HFD mice are due to the over-production of leptin, since mice treated with a soluble form of the leptin receptor do not develop high levels of MDSCs (79). However, MDSCs down-regulate leptin since mice depleted of MDSCs contain higher levels of leptin in their blood. Therefore, leptin levels drive the accumulation and function of MDSCs which enhance tumor progression by suppressing antitumor T cell responses. Interestingly, mice on a LFD have decreasing levels of MDSCs as their weight increases, suggesting that LFD is protective against increases in MDSCs, which is typically associated with weight gain (79).

Metabolic dysfunction in the form of elevated fasting glucose and increased insulin resistance is frequent in obese individuals and is characteristic of type 2 diabetes. As expected, Ob/Ob mice and mice on a HFD diet developed elevated fasting glucose levels and increased insulin resistance relative to Ob/+ mice and LFD mice, respectively. Unexpectedly, depletion of MDSCs from HFD mice significantly increased both insulin resistance and fasting glucose levels. Depletion of MDSCs from HFD mice also increased systemic and adipose tissue inflammation (IL-6 and TNF levels, respectively). However, HFD mice depleted of MDSC contained larger parametrial fat pads relative to non-depleted HFD mice. These studies demonstrate that although diet-induced MDSCs can accelerate tumor progression and metastatic disease, at the same time, they also protect against some of the metabolic dysfunction associated with obesity, while increasing adiposity and reducing the inflammation that accompanies adiposity (78). Therefore, in the setting of obesity and nutritional overload, MDSC play a beneficial role in counter-acting conditions that contribute to type 2 diabetes.

MDSCs in Pregnancy

During pregnancy the mother carries a semi-allogeneic fetus but does not mount an immune response against the embryo's histocompatibility or other antigens. This "maternal-fetal tolerance" has been ascribed to multiple mechanisms including immune suppressive T regulatory cells (Tregs) (81), tolerogenic dendritic cells (82), tryptophan catabolism by IDO (83), and several other factors. Several of these mechanisms are regulated

by MDSCs and early studies identified MDSC-like cells in pregnancy. Therefore, it was hypothesized that MDSCs may facilitate maternal-fetal tolerance. Pregnant women have high levels of Arg1 in their blood and in their placenta accompanied by down-regulation of the T cell receptor-associated CD3ζ chain and T cell hypo-responsiveness (84), characteristic effects of MDSCs. Cells with certain characteristics of MDSCs making Arg1, iNOS, and ROS are elevated at all stages of human pregnancy and decrease after parturition (85). Although the data on MDSCs in pregnant women is limited, these findings demonstrate that MDSCs are up-regulated during pregnancy and are consistent with a physiological requirement for MDSC for successful pregnancy (85, 86).

Studies in mice clearly demonstrate that MDSCs are essential for successful pregnancy (76–78). In pregnant mice immature myeloid cells analogous to cancer-induced MDSC accumulate in the placenta and produce the pro-angiogenic molecules matrix metalloproteinase-9 and Bv8 (76). Pregnancy-induced MDSCs tolerize via expression of activated STAT3 (78), and MDSC depletion and reconstitution studies identified implantation as a critical time for MDSC function and maintenance of maternal-fetal tolerance (77).

In addition to their direct effects on T effector cells, MDSC also indirectly impact T cells. Several of these indirect mechanisms have been implicated in inducing maternal-fetal tolerance. For example, maternal-fetal tolerance has been attributed to Tregs but MDSCs are known inducers of Tregs in the setting of cancer (87). This mechanism may well be active in pregnant women (88, 89). Studies of women with spontaneous miscarriages and elective abortions further support a critical role for MDSCs in successful pregnancy. Women experiencing early miscarriages have fewer immune suppressive MDSCs in their blood and endometrium relative to women who have delivered live babies (90). These findings have resulted in clinical trials to induce MDSCs in women with unexplained recurrent miscarriage, apparently with some reported success (91, 92). Collectively the observations in pregnant and aborting women combined with the mechanistic studies in mice demonstrate that MDSCs do play an essential normal physiological role in successful pregnancy by maintaining maternal-fetal tolerance.

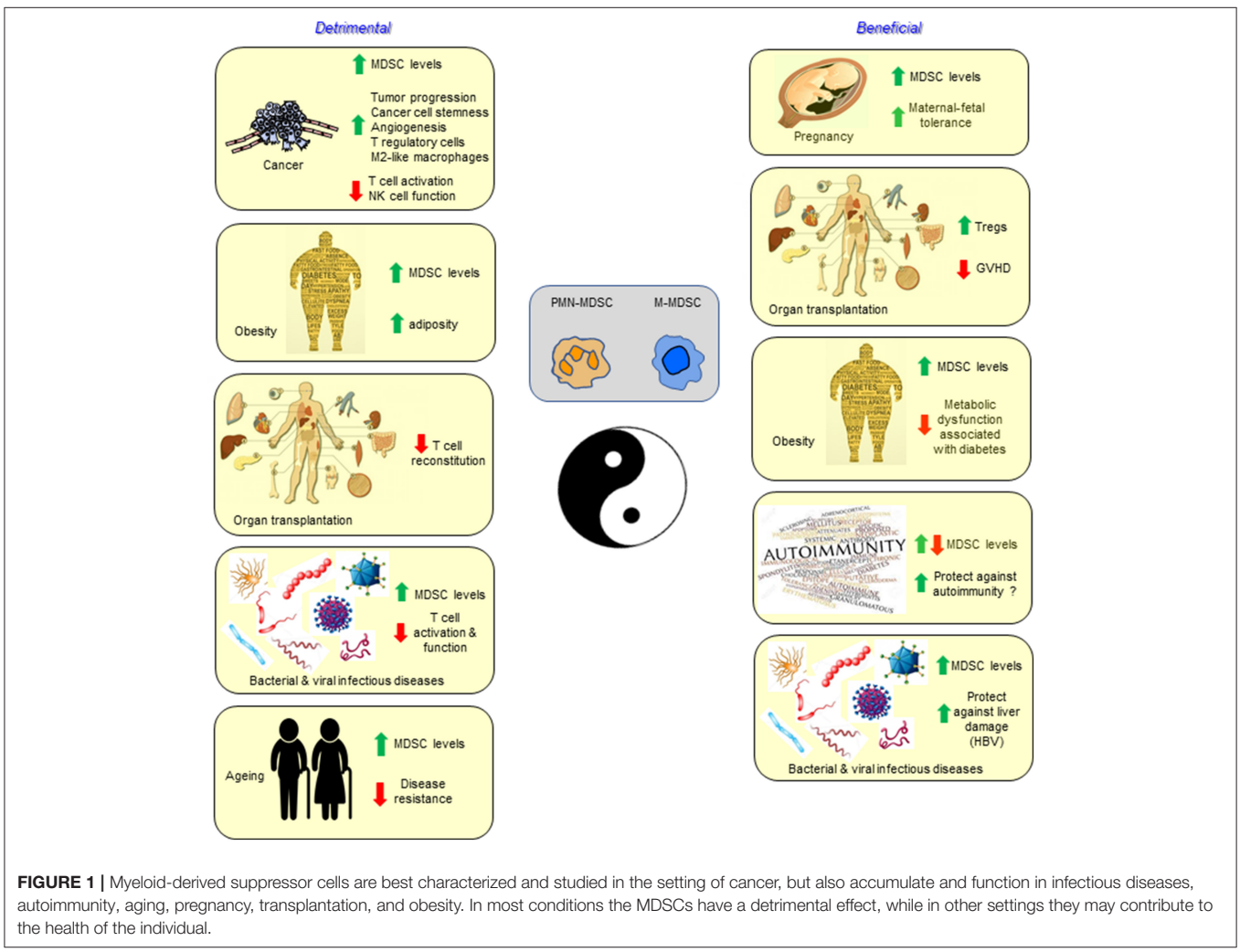

FIGURE 1 | Myeloid-derived suppressor cells are best characterized and studied in the setting of cancer, but also accumulate and function in infectious diseases, autoimmunity, aging, pregnancy, transplantation, and obesity. In most conditions the MDSCs have a detrimental effect, while in other settings they may contribute to the health of the individual.

MDSCs in Transplantation

In solid organ transplantation, mostly kidney, reports do suggest changes in levels of MDSCs after allografting (93). The expectation that higher levels of MDSCs might translate to better graft survival does seem to be borne out in several reports. For example, also in human renal transplantation, patients with higher MDSCs experienced less acute graft rejection, and maintained better graft function for a longer period of time (94). Factors influencing the relative levels of MDSCs, proportions of M-MDSCs-vs.-PMN-MDSCs, and the clinical implications of altered levels of these cells under immunosuppression following transplantation are now beginning to be explored (95).

MDSCs may not only directly inhibit effector T cells responsible for graft rejection, but also amplify Tregs (96). Interest in manipulating MDSCs to further transplantation tolerance in humans, as opposed to mouse models, is only recently becoming widespread, and most experience has been gained in cancer where efforts have been directed toward inhibiting MDSCs, not stimulating them (97). In mice, enhancing MDSC induction may confer benefit. For example in a skin transplant model, a combination of G-CSF and IL-2 coupled to an anti-IL 2 antibody increased MDSC (as well as Treg) levels and extended graft survival (98). In the naturally more tolerogenic human liver transplant setting, one mechanism by which tolerance is induced seems to be by stimulation of MDSCs (99). In a different clinical transplantation setting, the role of MDSCs has proven more equivocal. Although MDSCs may be beneficial in reducing graft-vs.-host disease (GVHD) in hematopoietic stem cell (HSC) transplantation, at the same time they can be inhibitory for T cell reconstitution and thus mediate negative effects [reviewed in (100)]. However, even in solid organ transplantation, MDSCs are a double-edged sword and can contribute to excessive immunosuppression (101). Nonetheless, efforts to control their induction not only by using agents known to enhance MDSCs in the cancer field (i.e., pro-inflammatory factors such as TNF (102), or G-CSF (103), or immune modulators such as dexamethasone (104), but also by novel approaches such as the use of cannabinoids (105), are ongoing. In murine HSC transplantation, a report of successfully applying *in vitro*-generated MDSCs to prevent GVHD at the same time allowing retention of CD8+ cytotoxic T cell effector function to maintain anti-cancer activity (106) raises hope that this outcome may also be achieved in humans and in solid organ transplantation.

CONCLUSIONS

The class of immune cells designated MDSCs is unequivocally important in dampening immunity in a wide range of cancers, and also in other pathological conditions involving chronic inflammation (**Figure 1**). However, there is also some evidence of a potentially beneficial effect in the iatrogenic situation of solid organ transplantation, as well as the parallel physiological "transplant" situation of pregnancy, and in combating some of the metabolic dysfunction associated with the pathology of obesity. We thus conclude that unlike Tregs, MDSCs are not likely to play a major role in the normal feedback control of immune responses with the single possible exception of their involvement in fetal tolerance.

AUTHOR CONTRIBUTIONS

GP, CV, and SO-R all drafted, reviewed, edited, and approved the manuscript.

REFERENCES

1. Wilcox RA. Cancer-associated myeloproliferation: old association, new therapeutic target. *Mayo Clin Proc.* (2010) 85:656–63. doi: 10.4065/mcp.2010.0077
2. Chinn IK, Blackburn CC, Manley NR, Sempowski GD. Changes in primary lymphoid organs with aging. *Semin Immunol.* (2012) 24:309–20. doi: 10.1016/j.smim.2012.04.005
3. Gabrilovich DI, Bronte V, Chen SH, Colombo MP, Ochoa A, Ostrand-Rosenberg S, et al. The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* (2007) 67:425; author reply: 6. doi: 10.1158/0008-5472.CAN-06-3037
4. Amodio G, Cichy J, Conde P, Matteoli G, Moreau A, Ochando J, et al. Role of myeloid regulatory cells (MRCs) in maintaining tissue homeostasis and promoting tolerance in autoimmunity, inflammatory disease and transplantation. *Cancer Immunol Immunother.* (2018) 68:661–72. doi: 10.1007/s00262-018-2264-3
5. Verschoor CP, Johnstone J, Millar J, Dorrington MG, Habibagahi M, Lelic A, et al. Blood CD33(+)HLA-DR(-) myeloid-derived suppressor cells are increased with age and a history of cancer. *J Leukoc Biol.* (2013) 93:633–7. doi: 10.1189/jlb.0912461
6. Alves AS, Ishimura ME, Duarte YAO, Bueno V. Parameters of the immune system and vitamin D levels in old individuals. *Front Immunol.* (2018) 9:1122. doi: 10.3389/fimmu.2018.01122
7. Mundy-Bosse BL, Thornton LM, Yang HC, Andersen BL, Carson WE. Psychological stress is associated with altered levels of myeloid-derived suppressor cells in breast cancer patients. *Cell Immunol.* (2011) 270:80–7. doi: 10.1016/j.cellimm.2011.04.003
8. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
9. Shipp C, Speigl L, Janssen N, Martens A, Pawelec G. A clinical and biological perspective of human myeloid-derived suppressor cells in cancer. *Cell Mol Life Sci.* (2016) 73:4043–61. doi: 10.1007/s00018-016-2278-y
10. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* (2012) 12:253–68. doi: 10.1038/nri3175
11. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol.* (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
12. Ostrand-Rosenberg S, Fenselau C. Myeloid-derived suppressor cells: immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J Immunol.* (2018) 200:422–31. doi: 10.4049/jimmunol.1701019
13. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer.* (2013) 13:739–52. doi: 10.1038/nrc3581
14. Ostrand-Rosenberg S. Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. *Cancer Immunol Immunother.* (2010) 59:1593–600. doi: 10.1007/s00262-010-0855-8
15. Trellakis S, Bruderek K, Hutte J, Elian M, Hoffmann TK, Lang S, et al. Granulocytic myeloid-derived suppressor cells are cryosensitive and

their frequency does not correlate with serum concentrations of colony-stimulating factors in head and neck cancer. *Innate Immun.* (2013) 19:328–36. doi: 10.1177/1753425912463618
16. Roussel M, Ferrell PBJr, Greenplate AR, Lhomme F, Le Gallou S, Diggins KE, et al. Mass cytometry deep phenotyping of human mononuclear phagocytes and myeloid-derived suppressor cells from human blood and bone marrow. *J Leukoc Biol.* (2017) 102:437–47. doi: 10.1189/jlb.5MA1116-457R
17. Wistuba-Hamprecht K, Martens A, Weide B, Teng KW, Zelba H, Guffart E, et al. Establishing high dimensional immune signatures from peripheral blood via mass cytometry in a discovery cohort of stage IV melanoma patients. *J Immunol.* (2017) 198:927–36. doi: 10.4049/jimmunol.1600875
18. Murray PJ. Immune regulation by monocytes. *Semin Immunol.* (2018) 35:12–8. doi: 10.1016/j.smim.2017.12.005
19. Mandruzzato S, Brandau S, Britten CM, Bronte V, Damuzzo V, Gouttefangeas C, et al. Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry: results from an interim study. *Cancer Immunol Immunother.* (2016) 65:161–9. doi: 10.1007/s00262-015-1782-5
20. Fridlender ZG, Sun J, Mishalian I, Singhal S, Cheng G, Kapoor V, et al. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PLoS ONE.* (2012) 7:e31524. doi: 10.1371/journal.pone.0031524
21. Petitprez F, Vano YA, Becht E, Giraldo NA, de Reynies A, Sautes-Fridman C, et al. Transcriptomic analysis of the tumor microenvironment to guide prognosis and immunotherapies. *Cancer Immunol Immunother.* (2018) 67:981–8. doi: 10.1007/s00262-017-2058-z
22. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol.* (2009) 182:4499–506. doi: 10.4049/jimmunol.0802740
23. Lechner MG, Liebertz DJ, Epstein AL. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *J Immunol.* (2010) 185:2273–84. doi: 10.4049/jimmunol.1000901
24. Lechner MG, Megiel C, Russell SM, Bingham B, Arger N, Woo T, et al. Functional characterization of human Cd33+ and Cd11b+ myeloid-derived suppressor cell subsets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines. *J Transl Med.* (2011) 9:90. doi: 10.1186/1479-5876-9-90
25. Mao Y, Poschke I, Wennerberg E, Pico de Coana Y, Egyhazi Brage S, Schultz I, et al. Melanoma-educated CD14+ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms. *Cancer Res.* (2013) 73:3877–87. doi: 10.1158/0008-5472.CAN-12-4115
26. Janssen N, Speigl L, Pawelec G, Niessner H, Shipp C. Inhibiting HSP90 prevents the induction of myeloid-derived suppressor cells by melanoma cells. *Cell Immunol.* (2018) 327:68–76. doi: 10.1016/j.cellimm.2018.02.012
27. Heine A, Held SAE, Schulte-Schrepping J, Wolff JFA, Klee K, Ulas T, et al. Generation and functional characterization of MDSC-like cells. *Oncoimmunology.* (2017) 6:e1295203. doi: 10.1080/2162402X.2017.1295203
28. Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood.* (2011) 118:5498–505. doi: 10.1182/blood-2011-07-365825
29. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res.* (2017) 5:3–8. doi: 10.1158/2326-6066.CIR-16-0297
30. Britten CM, Janetzki S, Butterfield LH, Ferrari G, Gouttefangeas C, Huber C, et al. T cell assays and MIATA: the essential minimum for maximum impact. *Immunity.* (2012) 37:1–2. doi: 10.1016/j.immuni.2012.07.010
31. Kanterman J, Sade-Feldman M, Biton M, Ish-Shalom E, Lasry A, Goldshtein A, et al. Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. *Cancer Res.* (2014) 74:6022–35. doi: 10.1158/0008-5472.CAN-14-0657
32. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, et al. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res.* (2006) 66:9299–307. doi: 10.1158/0008-5472.CAN-06-1690
33. Tobin RP, Jordan KR, Robinson WA, Davis D, Borges VF, Gonzalez R, et al. Targeting myeloid-derived suppressor cells using all-trans retinoic acid in melanoma patients treated with Ipilimumab. *Int Immunopharmacol.* (2018) 63:282–91. doi: 10.1016/j.intimp.2018.08.007
34. Liu Y, Wei G, Cheng WA, Dong Z, Sun H, Lee VY, et al. Targeting myeloid-derived suppressor cells for cancer immunotherapy. *Cancer Immunol Immunother.* (2018) 67:1181–95. doi: 10.1007/s00262-018-2175-3
35. Garber K. A new cancer immunotherapy suffers a setback. *Science.* (2018) 360:588. doi: 10.1126/science.360.6389.588
36. Beury DW, Carter KA, Nelson C, Sinha P, Hanson E, Nyandjo M, et al. Myeloid-derived suppressor cell survival and function are regulated by the transcription factor Nrf2. *J Immunol.* (2016) 196:3470–8. doi: 10.4049/jimmunol.1501785
37. Steding CE, Wu ST, Zhang Y, Jeng MH, Elzey BD, Kao C. The role of interleukin-12 on modulating myeloid-derived suppressor cells, increasing overall survival and reducing metastasis. *Immunology.* (2011) 133:221–38. doi: 10.1111/j.1365-2567.2011.03429.x
38. Jordan KR, Amaria RN, Ramirez O, Callihan EB, Gao D, Borakove M, et al. Myeloid-derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients. *Cancer Immunol Immunother.* (2013) 62:1711–22. doi: 10.1007/s00262-013-1475-x
39. Bailur JK, Gueckel B, Derhovanessian E, Pawelec G. Presence of circulating Her2-reactive CD8 + T-cells is associated with lower frequencies of myeloid-derived suppressor cells and regulatory T cells, and better survival in older breast cancer patients. *Breast Cancer Res.* (2015) 17:34. doi: 10.1186/s13058-015-0541-z
40. Vetsika EK, Koinis F, Gioulbasani M, Aggouraki D, Koutoulaki A, Skalidaki E, et al. A circulating subpopulation of monocytic myeloid-derived suppressor cells as an independent prognostic/predictive factor in untreated non-small lung cancer patients. *J Immunol Res.* (2014) 2014:659294. doi: 10.1155/2014/659294
41. Martens A, Zelba H, Garbe C, Pawelec G, Weide B. Monocytic myeloid-derived suppressor cells in advanced melanoma patients: indirect impact on prognosis through inhibition of tumor-specific T-cell responses? *Oncoimmunology.* (2014) 3:e27845. doi: 10.4161/onci.27845
42. Martens A, Wistuba-Hamprecht K, Yuan J, Postow MA, Wong P, Capone M, et al. Increases in absolute lymphocytes and circulating CD4+ and CD8+ T cells are associated with positive clinical outcome of melanoma patients treated with ipilimumab. *Clin Cancer Res.* (2016) 22:4848–58. doi: 10.1158/1078-0432.CCR-16-0249
43. Bakdash G, Buschow SI, Gorris MA, Halilovic A, Hato SV, Skold AE, et al. Expansion of a BDCA1+CD14+ myeloid cell population in melanoma patients may attenuate the efficacy of dendritic cell vaccines. *Cancer Res.* (2016) 76:4332–46. doi: 10.1158/0008-5472.CAN-15-1695
44. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Kruger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology.* (2008) 135:234–43. doi: 10.1053/j.gastro.2008.03.020
45. du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, et al. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after recent *Mycobacterium tuberculosis* infection suppresses T-cell function. *Am J Respir Crit Care Med.* (2013) 188:724–32. doi: 10.1164/rccm.201302-0249OC
46. Cai W, Qin A, Guo P, Yan D, Hu F, Yang Q, et al. Clinical significance and functional studies of myeloid-derived suppressor cells in chronic hepatitis C patients. *J Clin Immunol.* (2013) 33:798–808. doi: 10.1007/s10875-012-9861-2
47. Huang A, Zhang B, Yan W, Wang B, Wei H, Zhang F, et al. Myeloid-derived suppressor cells regulate immune response in patients with chronic hepatitis B virus infection through PD-1-induced IL-10. *J Immunol.* (2014) 193:5461–9. doi: 10.4049/jimmunol.1400849
48. Nonnenmann J, Stirner R, Roider J, Jung MC, Schrodl K, Bogner JR, et al. Lack of significant elevation of myeloid-derived suppressor cells in peripheral blood of chronically hepatitis C virus-infected individuals. *J Virol.* (2014) 88:7678–82. doi: 10.1128/JVI.00113-14
49. Dorhoi A, Glaria E, Garcia-Tellez T, Nieuwenhuizen NE, Zelinskyy G, Favier B, et al. MDSCs in infectious diseases: regulation, roles, and readjustment. *Cancer Immunol Immunother.* (2018) 68:673–85. doi: 10.1007/s00262-018-2277-y

50. Boros P, Ochando J, Zeher M. Myeloid derived suppressor cells and autoimmunity. *Hum Immunol.* (2016) 77:631–6. doi: 10.1016/j.humimm.2016.05.024
51. Kurko J, Vida A, Glant TT, Scanzello CR, Katz RS, Nair A, et al. Identification of myeloid-derived suppressor cells in the synovial fluid of patients with rheumatoid arthritis: a pilot study. *BMC Musculoskelet Disord.* (2014) 15:281. doi: 10.1186/1471-2474-15-281
52. Ioannou M, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, et al. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *J Immunol.* (2012) 188:1136–46. doi: 10.4049/jimmunol.1101816
53. Ballbach M, Hall T, Brand A, Neri D, Singh A, Schaefer I, et al. Induction of myeloid-derived suppressor cells in cryopyrin-associated periodic syndromes. *J Innate Immun.* (2016) 8:493–506. doi: 10.1159/000446615
54. Deshane JS, Redden DT, Zeng M, Spell ML, Zmijewski JW, Anderson JT, et al. Subsets of airway myeloid-derived regulatory cells distinguish mild asthma from chronic obstructive pulmonary disease. *J Allergy Clin Immunol.* (2015) 135:413–424 e15. doi: 10.1016/j.jaci.2014.08.040
55. White MC, Holman DM, Boehm JE, Peipins LA, Grossman M, Henley SJ. Age and cancer risk: a potentially modifiable relationship. *Am J Prev Med.* (2014) 46(3 Suppl 1):S7–15. doi: 10.1016/j.amepre.2013.10.029
56. Heithoff DM, Enioutina EY, Bareyan D, Daynes RA, Mahan MJ. Conditions that diminish myeloid-derived suppressor cell activities stimulate cross-protective immunity. *Infect Immun.* (2008) 76:5191–9. doi: 10.1128/IAI.00759-08
57. Enioutina EY, Bareyan D, Daynes RA. A role for immature myeloid cells in immune senescence. *J Immunol.* (2011) 186:697–707. doi: 10.4049/jimmunol.1002987
58. Jackaman C, Radley-Crabb HG, Soffe Z, Shavlakadze T, Grounds MD, Nelson DJ. Targeting macrophages rescues age-related immune deficiencies in C57BL/6J geriatric mice. *Aging Cell.* (2013) 12:345–57. doi: 10.1111/acel.12062
59. Hanson EM, Clements VK, Sinha P, Ilkovitch D, Ostrand-Rosenberg S. Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J Immunol.* (2009) 183:937–44. doi: 10.4049/jimmunol.0804253
60. Harman MF, Ranocchia RP, Gorlino CV, Sanchez Vallecillo MF, Castell SD, Crespo MI, et al. Expansion of myeloid-derived suppressor cells with arginase activity lasts longer in aged than in young mice after CpG-ODN plus IFA treatment. *Oncotarget.* (2015) 6:13448–61. doi: 10.18632/oncotarget.3626
61. Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci USA.* (2015) 112:E6301–10. doi: 10.1073/pnas.1515386112
62. Ruhland MK, Loza AJ, Capietto AH, Luo X, Knolhoff BL, Flanagan KC, et al. Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. *Nat Commun.* (2016) 7:11762. doi: 10.1038/ncomms11762
63. Fernandez IE, Greiffo FR, Frankenberger M, Bandres J, Heinzelmann K, Neurohr C, et al. Peripheral blood myeloid-derived suppressor cells reflect disease status in idiopathic pulmonary fibrosis. *Eur Respir J.* (2016) 48:1171–83. doi: 10.1183/13993003.01826-2015
64. Alvarez D, Cardenes N, Sellares J, Bueno M, Corey C, Hanumanthu VS, et al. IPF lung fibroblasts have a senescent phenotype. *Am J Physiol Lung Cell Mol Physiol.* (2017) 313:L1164–L73. doi: 10.1152/ajplung.00220.2017
65. Flores RR, Clauson CL, Cho J, Lee BC, McGowan SJ, Baker DJ, et al. Expansion of myeloid-derived suppressor cells with aging in the bone marrow of mice through a NF-kappaB-dependent mechanism. *Aging Cell.* (2017) 16:480–7. doi: 10.1111/acel.12571
66. Zhu J, Chen S, Wu L, Wang R, Zheng S, Zhao D, et al. The expansion of myeloid-derived suppressor cells is associated with joint inflammation in rheumatic patients with arthritis. *Biomed Res Int.* (2018) 2018:5474828. doi: 10.1155/2018/5474828
67. Le Page A, Garneau H, Dupuis G, Frost EH, Larbi A, Witkowski JM, et al. Differential phenotypes of myeloid-derived suppressor and T regulatory cells and cytokine levels in amnestic mild cognitive impairment subjects compared to mild alzheimer diseased patients. *Front Immunol.* (2017) 8:783. doi: 10.3389/fimmu.2017.00783
68. Thome AD, Faridar A, Beers DR, Thonhoff JR, Zhao W, Wen S, et al. Functional alterations of myeloid cells during the course of Alzheimer's disease. *Mol Neurodegener.* (2018) 13:61. doi: 10.1186/s13024-018-0293-1
69. Iacobaeus E, Douagi I, Jitschin R, Marcusson-Stahl M, Andren AT, Gavin C, et al. Phenotypic and functional alterations of myeloid-derived suppressor cells during the disease course of multiple sclerosis. *Immunol Cell Biol.* (2018) 96:820–30. doi: 10.1111/imcb.12042
70. Chen S, Liu Y, Niu Y, Xu Y, Zhou Q, Xu X, et al. Increased abundance of myeloid-derived suppressor cells and Th17 cells in peripheral blood of newly-diagnosed Parkinson's disease patients. *Neurosci Lett.* (2017) 648:21–5. doi: 10.1016/j.neulet.2017.03.045
71. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer.* (2004) 4:579–91. doi: 10.1038/nrc1408
72. Donohoe CL, Lysaght J, O'Sullivan J, Reynolds JV. Emerging concepts linking obesity with the hallmarks of cancer. *Trends Endocrinol Metab.* (2017) 28:46–62. doi: 10.1016/j.tem.2016.08.004
73. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell.* (2010) 140:883–99. doi: 10.1016/j.cell.2010.01.025
74. Font-Burgada J, Sun B, Karin M. Obesity and cancer: the oil that feeds the flame. *Cell Metab.* (2016) 23:48–62. doi: 10.1016/j.cmet.2015.12.015
75. Yan D, Yang Q, Shi M, Zhong L, Wu C, Meng T, et al. Polyunsaturated fatty acids promote the expansion of myeloid-derived suppressor cells by activating the JAK/STAT3 pathway. *Eur J Immunol.* (2013) 43:2943–55. doi: 10.1002/eji.201343472
76. Al-Khami AA, Zheng L, Del Valle L, Hossain F, Wyczechowska D, Zabaleta J, et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. *Oncoimmunology.* (2017) 6:e1344804. doi: 10.1080/2162402X.2017.1344804
77. Bao Y, Mo J, Ruan L, Li G. Increased monocytic CD14(+)HLADRlow/- myeloid-derived suppressor cells in obesity. *Mol Med Rep.* (2015) 11:2322–8. doi: 10.3892/mmr.2014.2927
78. Ostrand-Rosenberg S. Myeloid derived-suppressor cells: their role in cancer and obesity. *Curr Opin Immunol.* (2018) 51:68–75. doi: 10.1016/j.coi.2018.03.007
79. Clements VK, Long T, Long R, Figley C, Smith DMC, Ostrand-Rosenberg S. Frontline science: high fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. *J Leukoc Biol.* (2018) 103:395–407. doi: 10.1002/JLB.4HI0517-210R
80. Wang Z, Aguilar EG, Luna JI, Dunai C, Khuat LT, Le CT, et al. Paradoxical effects of obesity on T cell function during tumor progression and PD-1 checkpoint blockade. *Nat Med.* (2019) 25:141–51. doi: 10.1038/s41591-018-0221-5
81. Jiang TT, Chaturvedi V, Ertelt JM, Kinder JM, Clark DR, Valent AM, et al. Regulatory T cells: new keys for further unlocking the enigma of fetal tolerance and pregnancy complications. *J Immunol.* (2014) 192:4949–56. doi: 10.4049/jimmunol.1400498
82. Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science.* (2012) 336:1317–21. doi: 10.1126/science.1220030
83. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science.* (1998) 281:1191–3. doi: 10.1126/science.281.5380.1191
84. Kropf P, Baud D, Marshall SE, Munder M, Mosley A, Fuentes JM, et al. Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. *Eur J Immunol.* (2007) 37:935–45. doi: 10.1002/eji.200636542
85. Kostlin N, Kugel H, Spring B, Leiber A, Marme A, Henes M, et al. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur J Immunol.* (2014) 44:2582–91. doi: 10.1002/eji.201344200
86. Pan T, Liu Y, Zhong LM, Shi MH, Duan XB, Wu K, et al. Myeloid-derived suppressor cells are essential for maintaining feto-maternal immunotolerance via STAT3 signaling in mice. *J Leukoc Biol.* (2016) 100:499–511. doi: 10.1189/jlb.1A1015-481RR

87. Serafini P, Mgebroff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res*. (2008) 68:5439–49. doi: 10.1158/0008-5472.CAN-07-6621
88. Kang X, Zhang X, Liu Z, Xu H, Wang T, He L, et al. Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 expression in CD4+CD25-T cells by activation of the TGF-beta/beta-catenin pathway. *Mol Hum Reprod*. (2016) 22:499–511. doi: 10.1093/molehr/gaw026
89. Kostlin N, Vogelmann M, Spring B, Schwarz J, Feucht J, Hartel C, et al. Granulocytic myeloid-derived suppressor cells from human cord blood modulate T-helper cell response towards an anti-inflammatory phenotype. *Immunology*. (2017) 152:89–101. doi: 10.1111/imm.12751
90. Nair RR, Sinha P, Khanna A, Singh K. Reduced myeloid-derived suppressor cells in the blood and endometrium is associated with early miscarriage. *Am J Reprod Immunol*. (2015) 73:479–86. doi: 10.1111/aji.12351
91. Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. *Hum Reprod*. (2009) 24:2703–8. doi: 10.1093/humrep/dep240
92. Santjohanser C, Knieper C, Franz C, Hirv K, Meri O, Schleyer M, et al. Granulocyte-colony stimulating factor as treatment option in patients with recurrent miscarriage. *Arch Immunol Ther Exp*. (2013) 61:159–64. doi: 10.1007/s00005-012-0212-z
93. Hock BD, Mackenzie KA, Cross NB, Taylor KG, Currie MJ, Robinson BA, et al. Renal transplant recipients have elevated frequencies of circulating myeloid-derived suppressor cells. *Nephrol Dial Transplant*. (2012) 27:402–10. doi: 10.1093/ndt/gfr264
94. Meng F, Chen S, Guo X, Chen Z, Huang X, Lai Y, et al. Clinical significance of myeloid-derived suppressor cells in human renal transplantation with acute T cell-mediated rejection. *Inflammation*. (2014) 37:1799–805. doi: 10.1007/s10753-014-9910-5
95. Hock BD, McKenzie JL, Cross NB, Currie MJ. Dynamic changes in myeloid derived suppressor cell subsets following renal transplant: a prospective study. *Transpl Immunol*. (2015) 32:164–71. doi: 10.1016/j.trim.2015.05.001
96. Luan Y, Mosheir E, Menon MC, Wilson D, Woytovich C, Ochando J, et al. Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion. *Am J Transplant*. (2013) 13:3123–31. doi: 10.1111/ajt.12461
97. Scalea JR, Lee YS, Davila E, Bromberg JS. Myeloid-derived suppressor cells and their potential application in transplantation. *Transplantation*. (2018) 102:359–67. doi: 10.1097/TP.0000000000002022
98. Adeegbe D, Serafini P, Bronte V, Zoso A, Ricordi C, Inverardi L. *In vivo* induction of myeloid suppressor cells and CD4(+)Foxp3(+) T regulatory cells prolongs skin allograft survival in mice. *Cell Transplant*. (2011) 20:941–54. doi: 10.3727/096368910X540621
99. Moris D, Lu L, Qian S. Mechanisms of liver-induced tolerance. *Curr Opin Organ Transplant*. (2017) 22:71–8. doi: 10.1097/MOT.0000000000000380
100. Koehn BH, Blazar BR. Role of myeloid-derived suppressor cells in allogeneic hematopoietic cell transplantation. *J Leukoc Biol*. (2017) 102:335–41. doi: 10.1189/jlb.5MR1116-464R
101. Jiang Y, Feng S, Ji J, Lin Z, Zhang X. Persistent accumulation of circulating monocytic myeloid-derived suppressor cells contributes to post-infectious immunosuppression in renal transplant recipients with bacterial infection: a pilot study. *Transpl Immunol*. (2018) 48:10–7. doi: 10.1016/j.trim.2018.02.007
102. Yang F, Li Y, Wu T, Na N, Zhao Y, Li W, et al. TNFalpha-induced M-MDSCs promote transplant immune tolerance via nitric oxide. *J Mol Med*. (2016) 94:911–20. doi: 10.1007/s00109-016-1398-z
103. Luyckx A, Schouppe E, Rutgeerts O, Lenaerts C, Fevery S, Devos T, et al. G-CSF stem cell mobilization in human donors induces polymorphonuclear and mononuclear myeloid-derived suppressor cells. *Clin Immunol*. (2012) 143:83–7. doi: 10.1016/j.clim.2012.01.011
104. Liao J, Wang X, Bi Y, Shen B, Shao K, Yang H, et al. Dexamethasone potentiates myeloid-derived suppressor cell function in prolonging allograft survival through nitric oxide. *J Leukoc Biol*. (2014) 96:675–84. doi: 10.1189/jlb.2HI1113-611RR
105. Sido JM, Nagarkatti PS, Nagarkatti M. Delta(9)-Tetrahydrocannabinol attenuates allogeneic host-versus-graft response and delays skin graft rejection through activation of cannabinoid receptor 1 and induction of myeloid-derived suppressor cells. *J Leukoc Biol*. (2015) 98:435–47. doi: 10.1189/jlb.3A0115-030RR
106. Messmann JJ, Reisser T, Leithauser F, Lutz MB, Debatin KM, Strauss G. *In vitro*-generated MDSCs prevent murine GVHD by inducing type 2 T cells without disabling antitumor cytotoxicity. *Blood*. (2015) 126:1138–48. doi: 10.1182/blood-2015-01-624163

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The Reversal of Immune Exclusion Mediated by Tadalafil and an Anti-tumor Vaccine Also Induces PDL1 Upregulation in Recurrent Head and Neck Squamous Cell Carcinoma: Interim Analysis of a Phase I Clinical Trial

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Myeloid Derived suppressor cells (MDSCs) play a key role in the progression and recurrence of human malignancies and in restraining the efficacy of adjuvant therapies. We have previously shown that Tadalafil lowers MDSCs and regulatory T cells (Treg) in the blood and in the tumor, primes a tumor specific immune response, and increases the number of activated intratumoral $CD8^+$T cells in patients with primary Head and Neck Squamous Cell Carcinoma (HNSCC). However, despite these important immune modulatory actions, to date no clinically significant effects have been reported following PDE5 inhibition. Here we report for the first time interim results of our ongoing phase I clinical trial (NCT02544880) in patients with recurrent HNSCC to evaluate the safety of and immunological effects of combining Tadalafil with the antitumor vaccine composed of Mucin1 (MUC1) and polyICLC. The combined treatment of Tadalafil and MUC1/polyICLC vaccine was well-tolerated with no serious adverse events or treatment limiting toxicities. Immunologically, this trial also confirms the positive immunomodulation of Tadalafil in patients with recurrent HNSCC and suggests an adjuvant effect of the anti-tumor vaccine MUC1/polyICLC. Additionally, image cytometry analysis of scanned tumors indicates that the PDE5 inhibitor Tadalafil in conjunction with the MUC1/polyICLC vaccine effectively reduces the number of $PDL1^+$macrophages present at the tumor edge, and increases the number of activated tumor infiltrating T cells, suggesting reversion of immune exclusion. However, this analysis shows also that CD163 negative cells within the tumor upregulate PDL1 after treatment, suggesting the instauration of additional mechanisms

of immune evasion. In summary, our data confirm the safety and immunologic potential of PDE5 inhibition in HNSCC but also point to PDL1 as additional mechanism of tumor evasion. This supports the rationale for combining checkpoint and PDE5 inhibitors for the treatment of human malignancies.

Keywords: myeloid derived suppressor cells, tadalafil, PDE5, mucin 1 vaccine, poly-ICLC, recurrent HNSCC, PDL1, immune exclusion

INTRODUCTION

The incidence of head and neck squamous cell carcinoma (HNSCC) has declined in the last 30 years but this remains a deadly disease with more than 550,000 cases and 380,000 deaths reported annually worldwide (1). Despite advances in diagnostic imaging, surgical ablative, and complex reconstructive techniques, radiation therapy, and chemotherapy, recurrence remains high and outcomes often poor for advanced stage disease. Treatment of recurrent HNSCC is challenging because it is constrained by previous therapies that debilitate the patient and greatly modify the treatment field. Additional treatments for salvage impose significant morbidities with potentially little, or even detrimental, impact on outcome (2). The lower probability of long-term cancer control, combined with higher toxicity of current treatment modalities in this setting (advanced stage recurrence at a fully treated site), often makes cure a less central, or even unachievable, goal of patient care. For patients with resectable recurrent tumor, surgical salvage remains the first-line and often only available treatment in previously irradiated patients. Although the addition of chemoradiotherapy as re-irradiation to salvage surgery improved locoregional control and disease-free survival, no differences were observed in overall survival because of more treatment-related deaths, distant metastases, and second primary tumors among the re-irradiated patients (3).

The absence or the high morbidity of effective adjuvant treatments in patients with advanced recurrent HNSCC undergoing salvage surgery is the primary reason for the poor prognosis of this disease (44% 2 year recurrence-free survival all stages) (4), and clearly indicates the need for new treatments characterized by low morbidity profiles and improved efficacy. Cancer immunotherapy has become widespread in recent years and is often used as first line of treatment in both solid and hematological malignances (5). Immune checkpoint inhibitors, in particular, have demonstrated considerable promise for the treatment of melanoma, non-small cell lung cancer, and other cancers (6). In recurrent HNSCC, only immune checkpoint inhibitors have proven clinical efficacy in randomized phase III trials with Nivolumab (anti-PD1) being the only immunotherapeutic drug approved for platinum-refractory recurrent/metastatic HNSCC (7). Despite this promising development, however, response rates of recurrent/metastatic HNSCC to Pembrolizumab (anti-PDL1) or Nivolumab are low (16 and 16.9%, respectively) (8, 9). The use of checkpoint inhibitors for recurrent HNSCC undergoing salvage surgery is currently being evaluated to determine whether immune modulation before and after surgery can eliminate minimal residual disease and prevent tumor recurrence (10, 11).

Immune exclusion (also known as the absence of effector T cells inside the neoplastic lesion) is emerging as one of the main reasons that may explain the lack of response in patients undergoing immunotherapies such as checkpoint inhibition therapy (12, 13). Immune exclusion seems to be particularly important for patients with T3/T4 tumors undergoing salvage surgery because of the absence of tumor infiltrating $CD8^+$ T cells in ∼60% of the patients and the absence of PDL1 expression in more than 90% of the tumors (14). Although the lack of immunogenicity of the tumor may play a role, the polarization and phenotype of myeloid cells infiltrating the tumor and in circulation seems to be a major determinant. Indeed, an elevated ratio between both monocytic or granulocytic myeloid derived suppressor cells (MDSC) and lymphocytes in the periphery and at the tumor site is also emerging as an important predictive factor in the response to checkpoint inhibitors across different types of malignancies such as melanoma, HNSCC, and non-small-cell lung and genitourinary cancers (15–22). Considering the fact that MDSCs promote tumor growth not only by providing immune protection to the tumor but also by regulating tumor angiogenesis and metastasis (23, 24), safe therapeutic strategies aimed to inactivate, deplete, or convert these cells are highly desirable to further build on the success of checkpoint inhibitors and extend the number of patients that may benefit from immune therapeutic interventions. Indeed, in preclinical models, their functional inhibition is sufficient to restore the efficacy of anti-PDL1 antibodies (25). Furthermore, MDSCs and macrophages infiltrating the tumor express PDL1 and often are the major population in the tumor expressing this ligand (15). Thus, it is possible that strategies designed to eliminate/inhibit MDSCs and macrophages may even be sufficient to reverse T cell exhaustion and promote tumor rejection.

PDE5 inhibition, via repurposing of drugs commonly used for the treatment of erectile dysfunction, is an emerging experimental option that has been and is being tested in different clinical trials to lower MDSCs and prime or unleash the spontaneous anti-tumor immune response. In our original preclinical works, we showed that the PDE5 inhibitor sildenafil effectively inhibits MDSCs by increasing cGMP and reducing their expression of arginase 1, Nitric oxide synthase 2, and IL4Rα in mouse models of mammary carcinoma, colon cancer, and fibrosarcoma (26). PDE5 inhibition was sufficient to prime a spontaneous anti-tumor response, increase the number of tumor infiltrating T cells, and significantly decrease tumor progression (26). Furthermore, in a lymphoma model, we demonstrated that tumor progression and the accumulation of tumor specific

Treg accumulation correlated with the expression of IL4Rα in MDSCs (27). In this model, sildenafil, by lowering MDSCs activity, was sufficient to inhibit IL4Rα expression on MDSCs, reverse T cell anergy, and reduce the number of tumor specific Tregs (27). These data were then independently confirmed by different groups in colon carcinoma, spontaneous prostate cancer, melanoma, and metastatic mammary adenocarcinoma models (28–31).

In our previous double blinded, randomized, placebo controlled, phase1/2, independent clinical trials in HNSCC (NCT00894413, NCT00843635) (32, 33), Tadalafil was given daily pre-operatively for 14 (10 mg/day NCT00894413) or 21 (10 or 20 mg, NCT00843635) days. In both clinical trials, Tadalafil treatment was well-tolerated, with back pain and painful myalgias (all symptoms resolved within 48 h after treatment discontinuation) as a major side effect in a small percentage of subjects receiving the study drug. Analysis of cryopreserved PBMCs showed a significant reduction in both monocytic MDSCs and Treg (**Supplementary Figures 1A,B**) confirming in humans (32, 33) the immunomodulatory activity of Tadalafil observed in preclinical models (26, 27). Treatment was also associated with the reversal of systemic immunosuppression shown by a significant increase of the DTH response to recall antigens and upregulation of ζ-chain on $CD8^+$T cells (32). Furthermore, chronic PDE5 inhibition, significantly increased the anti-tumor T cell response evaluated by assessing the proliferation of magnetically purified CD3 T cells isolated before and after treatment to autologous dendritic cells pulsed with the autologous tumor (33). At the tumor site, treatment decreased MDSCs and Treg, and increased the number of $CD69^+$CTL and effector $CD4^+$ cells (33). Interestingly, further data analysis suggested that these positive effects were maximized at intermediate drug dosage (range 145–225 μg/Kg) possibly because of an off-target effect of Tadalafil on PDE11 at higher dose (33). However, despite these positive immunological effects and the surgical resection of the tumors, Tadalafil as monotherapy in a neoadjuvant setting, did not dramatically increase recurrence free survival in the treated patients as revealed by our analysis of the NCT00843635 trial (**Supplementary Figure 1C**).

Taken together these studies indicate that PDE5 inhibition positively modulates tumor immunity by reducing the systemic immunosuppression, by priming an anti-tumor immune response, and by increasing the infiltration of effector T cells in the tumor. However, to date, these studies have failed to demonstrate a dramatic clinical benefit of Tadalafil treatment in cancer patients.

Here we evaluate whether the combination of Tadalafil and an anti-mucin (MUC) 1 vaccine with poly ICLC as adjuvant is safe and can reverse immune exclusion in patients with recurrent stage 3 and 4 HNSCC as an interim analysis of a phase I clinical trial (NCT02544880). This trial is designed as a phase I lead-in in anticipation of a randomized phase II trial to compare the combination of Tadalafil and the anti MUC1/polyICLC vaccine with each therapy individually and in comparison with a non-randomized control group of patients undergoing surgical salvage alone.

MUC1 has been identified by the NCI as one of the top promising targets for cancer vaccines(34), as it is present in most of T2-T3 HNSCCs, and its expression is associated with tumor aggressiveness, lymph node metastases and a poor prognosis (35–41). While in normal tissues MUC1 is fully glycosylated and thus it is invisible to the immune system, in HNSCC this transmembrane protein is overexpressed and under-glycosylated (35–40). Importantly, MUC1 has been identified by a bead-based affinity-fractionated proteomic method as the immune dominant antigen for CD4 and CD8 T cells in 80% of patients with HNSCC (42). Clinical trials performed with MUC1 vaccines in patients with cancer showed an excellent safety profile with no sign of autoimmunity or serious side effects and encouraging results for less immunosuppressed patients (43–47). However, lack of response to the vaccine was observed in patients without cancer but with a history of premalignant lesions such as advanced colon adenomas and was further characterized by an elevated concentration of MDSCs in the blood of non-responding patients (47), suggesting a rationale for simultaneous PDE5 inhibition (to lower MDSCs) and anti-MUC1 vaccination.

MATERIALS AND METHODS

Clinical Trial Schema and Patient Enrollment

A phase I clinical trial (NCT02544880) is being conducted following the protocol approved by the IRB of the University of Miami and under the IND 16403. Patients undergoing salvage surgery with biopsy-proven, surgically resectable, recurrent or second primary HNSCC of the oral cavity, oropharynx, hypopharynx or larynx, recurrent stage III-IV, and whose recurrent tumors were within a previously irradiated field, were eligible for the trial. We excluded patients with distant metastatic disease, those that underwent prior immunotherapy with checkpoint inhibitors, those that used PDE5 inhibitors in the 2 weeks before enrollment, those with prior or known adverse reaction to PDE5 inhibitors, those immunocompromised for reasons not directly related to patient's malignancy, and those for which the study drugs are not recommended based on other clinical comorbidities. Additionally, to eliminate confounding variables, we excluded patients with hepatitis -B, -C or HIV, those with a history of severe autoimmune disease, female patients who were pregnant or breastfeeding, or patients in vulnerable subject categories. A complete list of inclusion and exclusion criteria is provided in **Supplementary Table 1**. This phase I trial was designed to accrue six evaluable patients as a lead-in for a three arm randomized phase II trial comparing the combination treatment of Tadalafil and the anti MUC1/poly ICLC vaccine with single modality treatment of either Tadalafil or the anti-MUC1/polyICLC vaccine, in addition to a fourth non-randomized control arm of otherwise eligible patients undergoing salvage surgery but unwilling to take study related drugs. The phase I lead-in and randomized phase II trials were designed to allow accrual to the non-randomized control arm to begin during the phase I lead-in should eligible patients for the control arm present during the enrollment period of the phase I

in order to maximize accrual to the control arm for the phase II trial. Eligible patients were enrolled to the non-randomized active treatment arm or as non-randomized controls after signing the appropriate IRB approved informed consent.

Following enrollment, patients in the experimental group were treated with Tadalafil for 19 days pre-operatively with anti-MUC1/poly ICLC vaccine given on day 7 of the Tadalafil course. Salvage surgery was performed 21 days following initiation of Tadalafil. Three additional courses of Tadalafil of 14 day durations with anti-MUC1/poly ICLC vaccine given on day 10 of each course were completed at ∼2, 4, and 6 months post-operative. A final anti-MUC1/ poly ICLC vaccine was given at 1 year post-operative. All patients were to be followed for 1 year beyond the end of course 5 or until withdrawn from the study for recurrence, TLT, death, or other reason. The study schema and flow chart is diagramed **Figure 1**.

For those patients assigned to the experimental group, enrollment was designed in a sequential fashion based upon Treatment Limiting Toxicities (TLTs) occurring during the first 2 Courses of treatment, in a manner such that no more than 2 patients were allowed to have TLTs at the same time. At least one of the first 2 patients enrolled must have been evaluated for TLT(s) up to the end of their Course 2 treatment, before patient 3 could begin Course 1 treatment. Patient 3 was allowed to begin Course 1 treatment if the first patient to complete evaluation for TLT's at the end of their Course 2 treatment did not experience a TLT. If neither patient 1 nor patient 2 experienced a TLT up to the end of both of their completion of Course 2 then patient 4 was allowed to begin Course 1 treatment. These same conditions applied for patients 5 and 6 beginning their Course 1 treatment (if no TLT was noted through completion of Course 2 for patients 1 and 2, and for either patient 3 or 4, then patient 5 would be allowed to begin Course 1, if neither patients 3 nor 4 experienced TLT after completion of both of their Course 2 treatments then patient 6 may begin Course 1 treatment). On the other hand if any patient experienced a TLT through the end of Course 2 then all subsequent patients would begin Course 1 treatment only after all prior patients had completed Course 2 with no additional TLTs identified.

Study subjects were considered evaluable for phase I safety analysis once they completed Course 2 or if they experienced a TLT prior to completion of Course 2. The phase I lead in was designed to accrue 6 evaluable patients for safety analysis. This safety analysis was planned following completion of Course 2 for the sixth evaluable study subject. An interim analysis of preliminary immunologic endpoints of all subjects enrolled in the phase I trial inclusive of those non-randomized controls enrolled during this same time period was planned to coincide with the safety analysis. Results of these combined analyses (safety and immunologic endpoints) were to be utilized to inform a decision whether or not to proceed with accrual to the randomized phase II trial. These results are presented below.

Monitoring for Adverse Events

The NCI common terminology criteria for adverse events (CTCAE3.0) were used to monitor toxicity. Laboratory monitoring, including CBC, BUN, creatinine, liver function tests, and ANA test were performed at baseline, before each treatment course and 14 days following course 4 and course 5, as well as 14 days following withdrawal from the study for other reasons such as recurrence or adverse event. Safety questionnaire was completed between 5 and 12 days following vaccination in each treatment course. A final safety questionnaire was administered at 24 months post-operative for those patients alive. Patients were questioned regarding adverse events with each follow up clinical evaluation for monitoring of their cancer status as per standard of care, including appropriate physical examination to assess disease status. Follow-up imaging was performed as clinically indicated. A treatment limiting toxicity (TLT) was defined as any one of the following adverse events (AEs) and was attributed (*possible, probable, or definite*) to the combination of Tadalafil/Vaccine treatment. Treatment discontinuation was required if a patient experienced a TLT. TLTs included new or worsening autoimmune disorder Grade $\geq$2, allergic reactions Grade $\geq$2 (Grade 2 drug fever considered an exception), and any other Grade $\geq$3 toxicity that in the opinion of the Investigator required discontinuation of study treatment. Exceptions included Grade $\geq$3 transient myalgia, back pain, or reversible hypotension, all of which were not considered a TLT if lasting <5 days. Patients were considered evaluable for safety who received at least one dose of Tadalafil, while patients were considered evaluable for TLTs who either experienced a TLT up to the end of Course 2 or received all scheduled doses of treatment through completion of Course 2 without TLT. The Sylvester Comprehensive Cancer Center (SCCC) Data and Safety Monitoring Committee (DSMC) monitored this clinical trial according to the Cancer Center's DSM Plan on a quarterly basis.

Specimen Collection

Blood (∼50 mL) was drawn in EDTA-containing tubes at baseline, at the day of surgery (after treatment); during tadalafil treatment on day 10/14 on course 2, 3, and 4; 15 days after treatment of course 4, and at course 5. Additional blood draws were performed at the exit visit either ∼15 days after course 5 or when a subject was withdrawn from the study because of recurrence, adverse events, or other reason. All specimens were processed within 2 h of being harvested. Fresh tumor specimen (at least 14 mm^3) was collected at the time of definitive tumor resection for tumor lysate preparation, and was processed within 1 h of harvesting. Additional specimens from available pretreatment biopsy and surgery were paraffin-embedded for immunofluorescence studies. For the control patients, blood was harvested before surgery and in eventual follow up visits.

Tadalafil Treatment and Dose

Tadalafil (Cialis™, Eli Lilly) was purchased through the UM clinical pharmacy and given orally q.d. at a weight-normalized dose as follow: 10 mg/day if weight $\leq$63.5 kg, 15 mg/day if weight >63.5 kg and $\leq$104.3 kg), or 20 mg/day for weight >104.3 kg as suggested in Weed et al. (33).

Vaccines and Immunization

Patients were immunized intramuscularly on day 7 of 20 in course 1, day 10 of 14 course 2–4, and day 1 of 1 in course 5 against MUC1 and, when seasonally available, influenza vaccine.

The MUC1 vaccine was composed of 50 μl of the MUC1 100 mer peptide (H2N-5X(GVTSAPDTRPAPGSTAPPAH-CONH2, [2 μg/μl], kindly provided as a gift by Dr. O. Finn, University of Pittsburg) admixed with 250 μl of POLY-ICLC (Hiltonol®, [2 μg/μl] provided by Oncovir at production cost) for total volume of 300 μl.

The influenza vaccine flublock, composed of recombinant proteins, was provided by Protein Sciences Corporation.

Dendritic Cells Preparation

Monocytes from freshly drawn PBMCs were isolated by adherence in a T75 flask (BD) for 2 h in RPMI-1640 containing 1% heat-inactivated human AB serum. Following washing to remove non-adherent cells, the adherent monocytes were differentiated into DC with RPMI-1640 1% AB serum containing 800 U/mL GM-CSF and 500 U/mL IL4 (Peprotech) for 5 days. Fresh GM-CSF and IL4 was added on day 3. On day 5, immature DC were transferred into 24-well plates and pulsed with MUC1 peptide (10 μg/mL) in RPMI-1640 1%AB serum supplemented with GM-CSF and IL4. Two hours later, pulsed immature DC were induce to mature by the addition of Mimic cytokine mix [5 ng/mL TNFα (Peprotech), 5 ng/mL IL1β (Peprotech), 750 ng/mL IL6 (Peprotech), and 1 μg/mL PGE2 (Sigma)].

Magnetic Sorting

$CD3^+$ T cells were purified by negative selection using the human Pan T Cell Isolation Kit II (Miltenyi Biotec) in combination with the LS column and following the manufacturer's instruction. Purity was evaluated by FACS and was generally higher than 90%.

Functional Assays

Magnetically purified, CFSE-labeled T cells (10^5) from baseline (Course 1 before treatment), or from 15 days after Course 4 were incubated with 3×10^5 autologous, monocyte-derived, DC pulsed with the MUC1 peptide. T-cell proliferation was evaluated by flow cytometry 4 days later.

Flow Cytometry

Flow cytometry was performed on whole blood and freshly ficolled PBMCs of patients at each time point. Data acquisition was performed on aBD LSRII equipped with the following wavelengths lasers: 405 nm (50 mW), 488 nm (50 mW), 532 nm (150 mW), and 640 nm (40 mW). MDSC phenotype analysis was performed using Zombie Violet™ Fixable Viability Dye (BioLegend) and the following anti-human Abs: CD33-FITC (clone HIM3-4; BD), Lox1-APC (clone 15C4; BioLegend), CD124-PE (clone 25463; R&D Systems), CD14-APC-H7 (clone MϕP9; BD), CD15-BV711 (clone W6D3; BD), HLA-DR V500 (clone G46-6; BD), CD11b-BV605 (clone ICRF44; BD). T-cell analysis was performed using Zombie Violet™ Fixable Viability Dye (BioLegend) with the following antibodies: CD3-Alexa Fluor 700 (clone OKT3; eBioscience), CD247-PE (clone 6B10.2; eBioscience), CD4-BV711 (clone SK3; BD), CD8-BV605 (clone SK1; BD), CD69-APC-Cy7 (clone FN50; BD), Foxp3-APC (clone 236A/E7; e-Bioscience), CD154-PE/Dazzle 594 (clone24-31; BioLegend). For the staining, 150 μl of whole blood or 5 × 105 ficolled PBMCs at 4°C, were admixed with 123-counting beads (e-bioscience) and the optimized antibodies cocktail for 30' at 4°C. Cells were washed with PBSand, lysed with 2 ml of ACK (Gibco) at RT for 15', washed twice with PBS, and labeleled with LIVE/DEAD staining. For T cell staining were then fixed and permeabilized and stained for Foxp3 using the Foxp3/Transcription Factor Staining Buffer Set (e-Bioscience) and following the manufacturer's instructions. Samples were read in the cytofluorimeter within 2 h of staining. At least 105 events were collected. Compensation was performed using compi-beads (BD) after data collection. FMO were used as negative controls. Data were analyzed using the FCS vs6 (*denovo* software). Gating strategy are summarized in **Supplementary Figure 3**.

MUC1 IHC

IHC was performed as described in Cascio et al. (48). Briefly, deparaffinized and rehydrated 4 μm sections of tumor specimen were incubated for 15 min at RT in a 30%H_2O_2/methanol solution (1:10) to block endogenous peroxidase activity. Slide were washed 3 times with PBS 1X, antigens were retrieved in 0.1% citrate buffer pH 6 for 5'at 120°C. Sections were permeabilized in PBS-0.2% Tween20 (5'at RT)and incubated with incubated PBS-2%BSA (20' at RT) to block non-specific binding. Samples were then incubated 1 h RT with a 1:40 dilution in PBS−2% BSA of the anti Mucin 1 antibody that specifically recognizes the underglycosylated, tumor specific form, of MUC1 (VU-4H5, Santa Cruz Biotechnology). Slides were washed in PBS-0.2% TWEEN20 (5'at RT) and incubated for 1 h with the biotinylated anti-mouse IgG secondary antibody (Vector Laboratories dilution 1:200 in PBS-BSA2%) and washed in PBS-0.2% TWEEN 20 for 5'at RT. Slices were incubated with ABC solution (Vector Laboratories) for 30'at RT washed, developed with DAB substrate (BD Pharmingen).

Image Cytometry

Four μm sections of tumor specimen were deparaffinized, rehydrated, and incubated for 30 min at RT in a sodium borohydride solution (0.5 mg/mL in PBS; EMDGibbstown, NJ, USA) to reduce auto fluorescence. Antigen retrieval was performed by a 5 min incubation at 120°C in EDTA antigen retrieval solution pH = 9 (GIBCO Carlsbad, CA, USA). Slides were then incubated with Image-iT (Invitrogen) for 30 min at RT followed by incubation (1 h at RT) with PBS containing 1% BSA and 0.05% Triton-X100 to permeabilize the tissue and block non-specific binding. Samples were incubated O/N at 4°C with the primary antibodies diluted in PBS with 1% BSA. After three washes with PBS, samples were labeled for 2 h at RT with the relevant secondary antibodies, counterstained in PBS containing 2 mM DAPI (Invitrogen), for 30 min at RT, and rinsed with PBS. Coverslips were mounted using Biomeda gel mounting media (Electron Microscopy sciences, Hatfield, PA, USA). The following primary antibodies were used: mouse monoclonal anti-human FOXP3 antibody (clone 237/E7, dilution 1/25, Abcam) and the goat polyclonal anti-human CD4 antibody,

(dilution 1/20, R&D System). Rabbit polyclonal anti-human CD33 antibody (dilution 1/15, Santa Cruz Biotechnology) and mouse monoclonal anti-human IL4Rα antibody (clone 25463, dilution 1/15, R&D System). Rabbit polyclonal anti-human CD8 antibody (dilution 1/30, Abcam) and goat polyclonal anti-human CD69 antibody (dilution 1/25, R&D System). Mouse monoclonal anti-human CD163 antibody (clone 10D6 dilution 1/100, Leica Biosystem) and rabbit monoclonal anti-human PD-L1 antibody (clone SP142, dilution 1/50, Abcam). As secondary antibodies we used: Alexa Fluor-555 conjugated anti-mouse antibody (for FoxP3, CD163 and IL4Ra, Invitrogen); Alexa Fluor-488 conjugated anti-goat antibody (for CD4 and CD69, Invitrogen); Alexa Fluor-555 conjugated anti-rabbit antibody (for CD8, Invitrogen); Alexa Fluor-488 conjugated anti-rabbit antibody (for CD33 and PD-L1, Invitrogen) all secondary antibodies were diluted 1/500 in PBS/BSA 1%. Stained slides were scanned at 20X with an Olympus VS120 microscope (Olympus) using a DAPI CUBE 455 nm, a FITC CUBE 518 nm and a TRITC CUBE 580 nm. Images for each patient were exported as single channel tiff files with OlyVIA software with a resolution of 5x and qualitatively evaluated with ImageJ (https://fiji.sc/) and processed with cell-profiler (www.cellprofiler.com) and fed into FCS Express 6 plus (https://www.denovosoftware.com/site/Plus-Overview.shtml). Detailed on image processing are provided in **Supplementary Material** section.

ELISA

IgG, IgA, and IgM levels were examined in the plasma of patients for each time point as previously described (47). Briefly, Immulon plates were coated with 1 μg/well of MUC1 peptide or recombinant influenza proteins (Flublock) and incubated overnight at 4°C. The next day, plates were washed and then incubated at room temperature (RT) for 1 h with blocking buffer (DPBS-BSA 2.5%). Blocking buffer was discarded and 50 μl of plasma (diluted 1:40 in blocking buffer) was added to the plates (in duplicates) and incubated at RT for 1 h. After another washing step, 50 μl of diluted goat anti Human-HRP IgA; IgG or IGM secondary antibody (for MUC1) or a combination of the three antibodies (HA) was added to each well, and plates were incubated at RT for 1 h. Plates were washed and a 100 μl of substrate solution (SIGMA-FAST p-Nitrophenyl) was added to each well and plates were incubated for 20 min at RT followed by the addition of 50 μl of stop solution (NaOH 0.5 M). Absorbance was measured at 405 and 410 nm wavelength.

Statistical Analysis

Statistical analysis was performed in coordination with the statistical core at the Sylvester cancer center. Time comparison within a treatment arm was assessed by paired *t*-test or RM-one way ANOVA. Comparisons between treatment arms were done by two-sample *t*-tests or ANOVA, or by non-parametric methods, the Mann–Whitney or Kruskal–Wallis test. All tests were two-sided with 5% significance statistical analysis. An interim safety analysis was planned after the 6th patient in phase I completed Course 2 (of planned 5 treatment courses). This interim analysis was planned to evaluate safety with attention to occurrence of TLTs, other AEs, as well as clinical data such as recurrence, and preliminary analysis of immunologic endpoints. The results of this interim analysis are summarized in this report. Recurrence-Free Survival (RFS) was evaluated for RFS by follow-up assessment(s) post-surgery as per routine care. RFS is defined as the time from date of Surgery to the date of first documented recurrence. Recurrence was demonstrated by clinical assessments such as clinical examinations and tumor assessments (possibly) by CT, PET/CT or MRI. Patients under follow-up and those lost to follow-up have been censored at the last date of documented recurrence-free status. Correlations were evaluated by Pearson correlation analysis. Statistical analyses were conducted using SAS software version 9.2 (SAS) or Sigmaplot vs12.5.

RESULTS

Underglycosylated Muc1 Is a Tumor-Associated Antigen Widely Expressed in Patients With Recurrent HNSCC

Underglycosylated MUC1 has been proposed as a tumor associated antigen in HNSCC, however, its expression in recurrent HNSCC has not been analyzed. Thus, we performed IHC on the tumor specimens and each staining was independently scored from 0 (no staining) to 4 (strong homogenous staining) by four experienced investigators. Scores were averaged, and examples of staining and relative scores are reported in **Supplementary Figure 2**. Underglycosylated MUC1 was found expressed in most of the analyzed specimens whereas it was undetectable in the "normal" tissue surrounding the neoplastic lesions (**Figures 2A,B**). This analysis supports the notion of underglycosylated MUC1 as a tumor specific antigen in patients with recurrent HNSCC. We next evaluated whether a preexisting immunity was present in these patients as well as in healthy donors. Anti-MUC1 IgG antibodies were significantly higher in the sera of recurrent HNSCC patients compared to one of age matched healthy controls (**Figure 2C**) suggesting the presence of a memory response against this antigen. Taken together, these data suggest that underglycosylated MUC1 is an immunogenic tumor specific antigen in recurrent HNSCC.

Tadalafil and MUC1/polyICLC Vaccine Are Well-Tolerated in Patients With Recurrent HNSCC

Although MUC1 vaccine and PDE5 inhibitors has been proven safe when used as monotherapy in cancer patient the safety of the combination of these two immunologic strategies has not been previously evaluated. Thus, we performed a proof of principle, phase I clinical trial (NCT02544880) with safety and immunological endpoints in patients with recurrent stage 3 and 4 HNSCC undergoing salvage surgery (**Figure 1**). As controls we enrolled eligible patients willing to donate blood and tumor specimen but unwilling to receive study drugs. This cohort of patients was chosen because of the absence or the high morbidity of effective non-surgical treatments as alternatives to or adjuvant to standard of care salvage surgery, and the high recurrence rate (up to 70%) associated with salvage surgery alone in patients with

recurrent, resectable, advanced staged HNSCC in a previously irradiated field (49). Even a small trial in this population might provide some insights regarding the clinical efficacy of an experimental treatment. Patient demographics, clinical characteristics, and complete list of inclusion and exclusion criteria are reported in **Supplementary Tables 1, 2**.

In this trial, patients received 4 courses of Tadalafil (orally q.d.) in association with MUC1/polyICLC vaccine (i.m. 1 week after each Tadalafil treatment initiation). The first course (19 days) was given in a neoadjuvant setting whereas course 2, 3, and 4 (14 days each) were given ∼5, 12, and 21 weeks after salvage surgery. One year after surgery (course 5), patients received the MUC1/polyICLC vaccine without Tadalafil.

A total of 14 patients have been enrolled on this trial, 6 as control and 8 patients to the active treatment arm. Two patients on the active treatment arm were not evaluable for treatment limiting toxicity (TLT) analysis. Subjects were evaluable for TLT if they completed at least two courses of study drug or if they developed a TLT at any time prior to completion of course 2. One (subject 1–01) developed disease progression following early tumor recurrence after lengthy recovery from surgery and was withdrawn from the study without receiving course 2 study treatment. The other patient was non-compliant with study drug administration and was withdrawn at the beginning of course 1. Disease sites for 6 evaluable treatment patients included oral cavity ($n = 2$), oropharynx ($n = 2$), and larynx ($n = 2$), while disease sites of control patients included oral cavity ($n = 4$), oropharynx ($n = 1$), and larynx ($n = 1$). Recurrent tumor summary stage for active treatment patients were IV ($n = 5$) and III ($n = 1$), while all control patient recurrent summary stage was IV ($n = 6$). Three of the 12 enrolled patients had p16 positive tumors consistent with HPV related malignancies, including 1 control and 2 treatment patients. All patients were previously irradiated as per study protocol, with 4 of 6 on the active treatment arm and 5 of 6 on the control arm receiving chemotherapy with radiation therapy as part of their prior treatment.

A total of 27 grade 1 or 2 adverse events (AEs) were recorded in the 8 phase 1 patients who received any study drug (**Supplementary Table 3**) and included flushing (1), headache (3), myalgia (1), nausea (1), vomiting (1), and an asymptomatic autoimmune disorder (1) as revealed by the development of anti-nuclear antibodies (ANA) while on treatment. The subject was electively withdrawn from the active treatment arm of the trial, and subsequent ANA testing of this subject has reverted to normal. No TLTs were recorded in the 6 patients evaluable for TLT. One of 6 evaluable active treatment patients continues to receive treatment on trial, having just completed course 4 with no evidence of recurrence. One of the remaining 5 active treatment patients completed all treatment courses but developed recurrence in the second year of follow-up. One of the remaining 5 active treatment patients was withdrawn from the study due to the development of a positive ANA, with no evidence of recurrence. The remaining three active treatment patients developed recurrence prior to completion of all study courses. Thus, far overall recurrence free survival of treated and control patients is similar (**Supplementary Figure 6**) but should not be relied upon given the small number of patients and ongoing follow-up of the clinical trial. In summary, the study cohort of heavily pre-treated advanced recurrent-staged tumors thus far exhibits an expected high rate of recurrence. The study treatment has been well-tolerated with minimal side effects.

Tadalafil and MUC1 Vaccine Decrease MDSCs and Treg in the Peripheral Blood and Restore the Expression of CD3 ζ-Chain in the CD8$^+$T Cells

Longitudinal Immunomonitoring was performed on the peripheral blood to assess the changes in monocytic MDSC [mMDSC defined as $CD33^+IL4R\alpha^+CD14^+CD11b^+HLADR^{low/-}$cells (33)], granulocytic MDSC [gMDSC defined as $CD33^+IL4R\alpha^+CD15^+CD11b^+HLADR^{low/--}Lox1^+$ cells (50)], and regulatory T cells (Treg defined as $CD3^+CD4^+Foxp3^+$T cells) (**Supplementary Figure 3**). Additionally, we evaluated the expression of CD3 ζ-chain in the CD8$^+$T cells since its down-regulation is associated with MDSCs activity, T cell apoptosis, disease stage, and worse prognosis in patients with HNSCC (51–53). As expected, compared to age matched healthy controls, mMDSC, gMDSC, and Treg were significantly increased in patients with recurrent stage 3 and 4 HNSCC whereas CD3 ζ-chain in the CD8$^+$T cells (**Figure 3A**) was downregulated.

Even before salvage surgery, Tadalafil treatment (gray shadowed area) significantly lowered both MDSC subsets and Treg and increased the expression of CD3 ζ-chain in the CD8$^+$T cells. These positive modulations were maintained during the treatment in course 2, 3, and 4 (**Figure 3B**). Interestingly, 15 days after Tadalafil termination in course 4, an increased in gMDSCs and mMDSC and a decrease in ζ-chain expression was observed in many patients suggesting that active mechanisms of MDSCs expansion were still present even without any clinical detectable tumor (**Figure 3B**). Taken together, these results confirm a beneficial action of Tadalafil and possibly MUC1/polyICLC vaccine to the tumor macro-environment. However, these effects are reverted upon treatment discontinuation, possibly suggesting the presence of a microscopic disease being present prior to its becoming clinically evident.

We then evaluated the capacity of the patients to mount an immune response to tumor associated antigen (MUC1) or unrelated antigens (recombinant flu antigens, flublock vaccine). To accomplish this aim patients that enrolled in the treatment arm received the MUC1 peptide vaccine admixed to polyICLC as adjuvant (intramuscular in the right arm) and the flublock vaccine (intramuscular in the left arm when seasonally available) on day 7 of course 1, on day 10 of course 2, 3 and 4, and on day 0 of course 5. Response to vaccines was evaluated by ELISA on the serum to determine the concentration of IgM, IgG, and IgA against the MUC1 or the influenza antigens. The choice of these assays was determined by their simplicity, HLA type independence, and by the fact that the presence of IgG antibodies against a tumor associated antigen correlates well with the CTL response (54). Longitudinal analysis of the treated patients reveals a higher titer of IgM or IgA antibodies against influenza

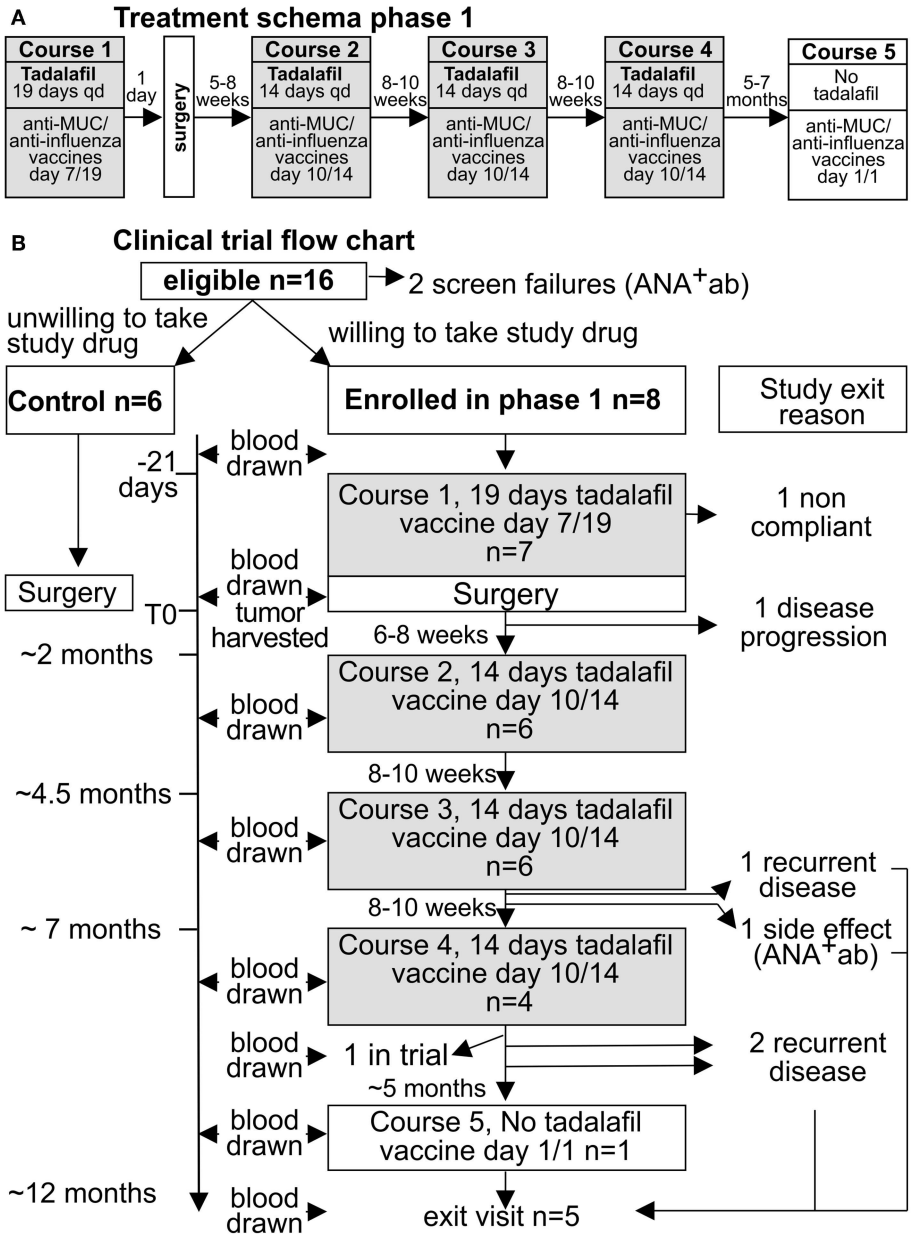

FIGURE 1 | Flow diagram and design of the phase 1 study.

antigens in 5 out of 6 patients following vaccination (**Figure 4A**). In contrast, only two patients showed a significant immune response against the MUC1 vaccine (**Figure 4A**). Interestingly, the responses to the MUC1 vaccine were observed only in the patients that had not received chemotherapy in conjunction with their radiation treatment for their original cancer treatment prior to recurrence.

To evaluate whether treatment could increase T cell mediated immunity against MUC1, magnetically purified $CD3^+$ T cells, harvested before treatment initiation (C0) and 2 weeks after course 4 (C4), were stimulated with autologous DCs pulsed with the MUC1 peptide or left unpulsed. $CD8^+$ and $CD4^+$ T cell proliferation was evaluated 4 days later by flow cytometry (**Figure 4B**). Compared to baseline, an increase response to the relevant peptide (up to 2–3% of proliferating T cells within the CD4 or CD8 populations) was detected after the 4 courses of treatment in 3 of the 4 evaluated patients.

Taken together these data suggest that Tadalafil and MUC1/polyICLC vaccine positively modulate the immune system systemically in patients with recurrent HNSCC undergoing salvage surgery. However, a strong memory

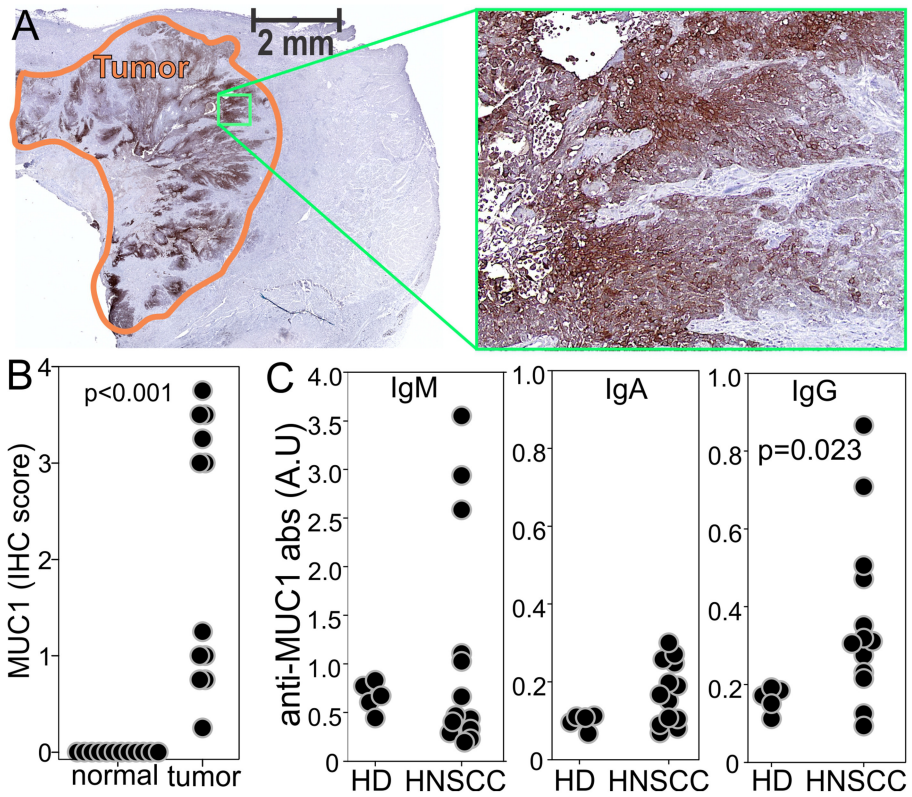

FIGURE 2 | Underglycosylated MUC1 is a common tumor specific antigen in stage 3/4 recurrent HNSCC. **(A)** MUC1 was evaluated by IHC on slides from the tumor specimens of enrolled patients. **(B)** Tumors and surrounding "normal" tissues were scored from 0 to 4 independently by four investigators based on the staining intensity and extension, and individual scores averaged. Examples of different scores are reported in **Supplementary Figure 2**. **(C)** ELISA for MUC1 specific antibodies was performed on the sera of healthy donors or enrolled patients at baseline.

IgG immune response against underglycosylated MUC1 is detectable only in a fraction of the patients.

Tadalafil and MUC1/polyIC Treatment Lowers MDSCs and Treg at the Tumor Site and Reverse Immune Exclusion

Immunofluorescence based image cytometry was employed to determine the effect of treatment at the tumor site both objectively and topographically (**Supplementary Figure 4**). Briefly, slides from the surgical specimens were stained to identify $CD33^{+}IL4R\alpha^{+}$MDSCs (33), $CD4^{+}$Treg expressing Foxp3 in the nucleus (55), or activated $CD8^{+}CD69^{+}$ T cells. Stained slides were acquired with a high resolution microscanner, processed with cell-profiler to identify each individual cell, and fed into FCS-image express to enumerate the cell of interest and the expression of a particular protein. This process allowed for analysis of 10^{5}-10^{6} cells inside the tumor, at the tumor edge, and in "normal" adjacent tissue as defined by an experienced pathologist in serial H&E slides. Compared to the untreated controls, $CD33^{+}IL4R\alpha^{+}$MDSCs were significantly lower inside the tumor in the treated patients (**Figure 5A**), whereas no differences were found at the tumor edge or in the "normal" tissue nor in the total number of $CD33^{+}IL4R\alpha^{-}$ myeloid cells (**Figure 5B** and **Supplementary Figure 5**). Similarly, a lower concentration of Tregs with nuclear FoxP3 was found in the tumor of treated patients compared to controls whereas no differences were detected in the naïve ($CD4^{+}Foxp3^{-}$cells) or poorly activated [$CD4^{+}$cells with cytoplasmic Foxp3 (55, 56)] $CD4^{+}$T cells (**Figure 5C**). Conversely, a higher number of $CD8^{+}$T cells were found in the tumor of treated patients compared to the controls whereas no differences were found in the tumor edge and in the surrounding normal tissue (**Figure 5D** and **Supplementary Figure 4E**). Furthermore, analysis of CD69 indicated a significantly higher expression of this early activation marker in the $CD8^{+}$cells (**Figure 5E** and **Supplementary Figure 4F**) of the treated patients compared to controls. Interestingly, the expression of CD69 significantly correlates with the MUC1 expression in the same specimen determined by IHC in the treated patients whereas no correlation was observed in the untreated controls (**Figure 5F**). This suggests that despite the poor immune response against MUC1 detected in most patients (**Figure 4**), an immune response against this tumor associated antigen has been primed and resulted in the infiltration of activated CTL at the tumor site.

Taken together, these results indicate that Tadalafil and polyICLC/MUC1 vaccine reshape the tumor microenvironment,

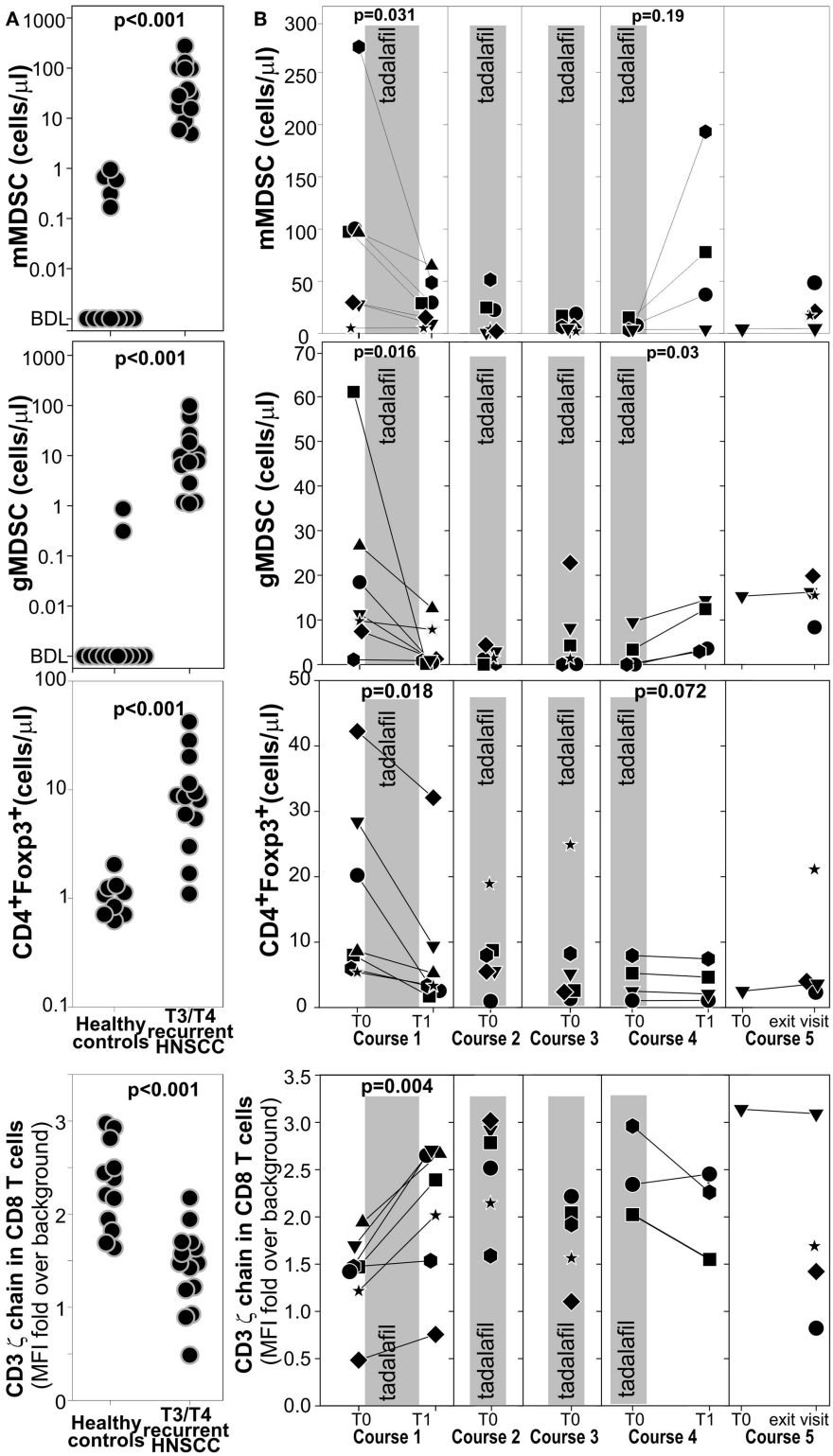

FIGURE 3 | Tadalafil and MUC1/polyICLC vaccine lowers circulating MDSCs and Treg and restores the expression of CD3 ζ-chain expression on CD8 T cells**. (A)** mMDSC, gMDSC, Treg, and the expression of CD3 ζ-chain expression on CD8 T cells was evaluated by multicolor flow cytometry on fresh blood of the enrolled patients or on age matched healthy donors. See **Supplementary Figure 3** for gating strategies. Leukocyte subsets were enumerated with "123 beads" Two ways *T*-test value are reported. **(B)** The same subsets as in A were evaluated longitudinally in the patients enrolled in the treatment arm. The gray area correspond to the Tadalafil treatment. Significant Paired *T*-test value are indicated.

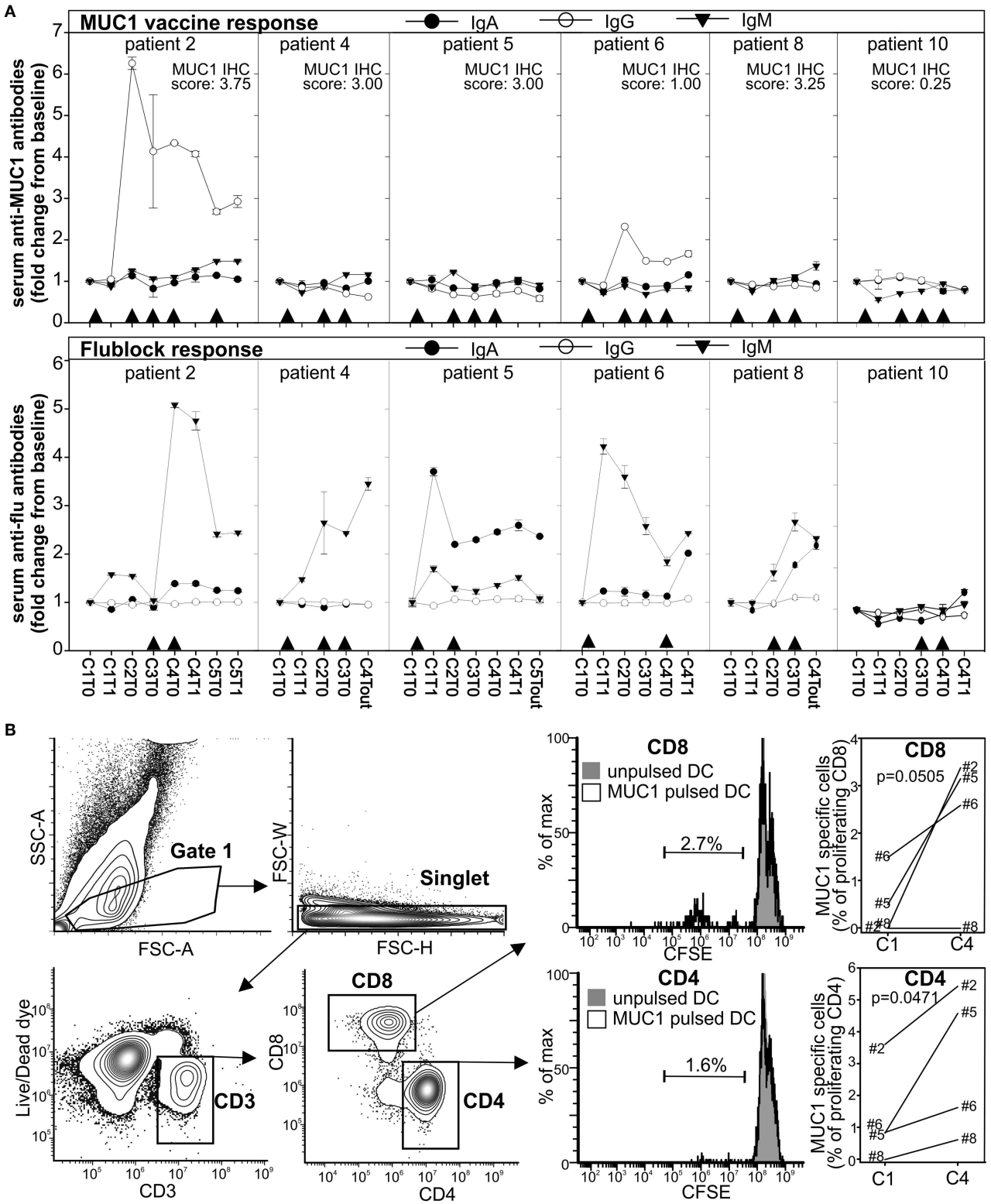

FIGURE 4 | Immune response to the MUC1 and the influenza vaccines. **(A)** Anti-MUC1 or anti-flublock antibodies were evaluated longitudinally by ELISA in the plasma of the patients in the treatment arm. Arrowheads indicate the immunization time. MUC1 IHC score is indicated. **(B)** T cells from PBMCs drawn at baseline (before treatment initiation of course 1) and 2 weeks after completion of course 4 were stimulated with monocytes-derived autologous DC pulsed with MUC1 peptide. Four days later, $CD8^+$ T-cell proliferation was evaluated by FACS. Background from parallel culture using unpulsed DC was subtracted.

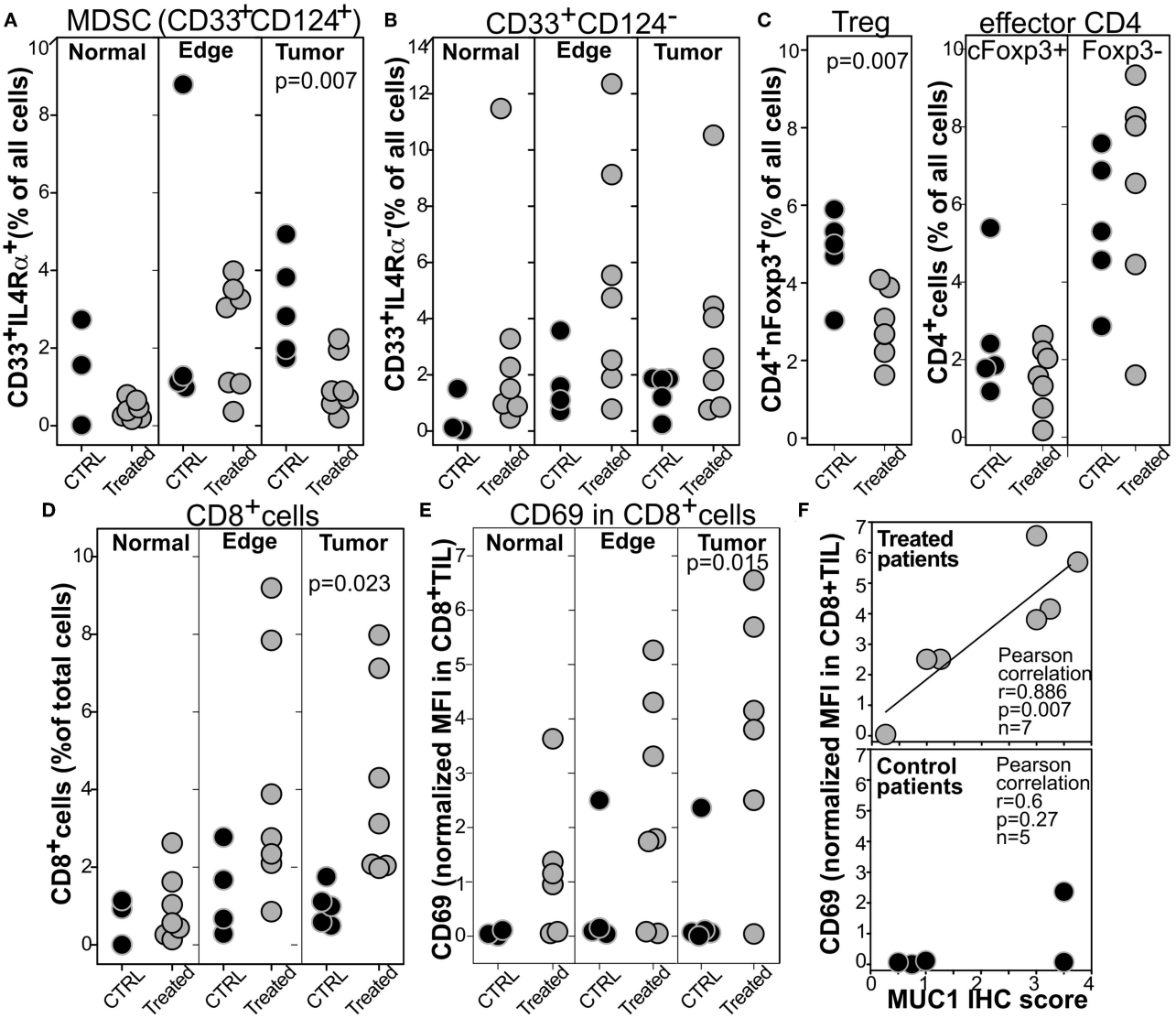

FIGURE 5 | Tumors from patients treated with Tadalafil and MUC1/polyICLC vaccine show a lower infiltration of MDSCs and Treg and a higher infiltration of activated CD8 in the tumor bed. Computer based image cytometry was performed to enumerate the number of **(A)** MDSC, **(B)** IL4Rα$^-$myeloid cells, **(C)** CD4$^+$T cell subsets, or **(D)** CD8$^+$T cells. **(E)** CD69 expression within the CD8 is reported normalized on the CD69 expression on all the cells evaluated. Depending on the region of interest evaluated, at least 10^5-10^6 cells were analyzed. **(F)** The expression of CD69 in CD8$^+$T cells was plotted against MUC1 IHC score of the corresponding tumor. Two ways *T*-test and relevant pearson correlation parameters are reported.

lowering the immune suppressive populations and increasing the number of activated T cells.

Reversion of Immune Exclusion by Tadalafil and MUC1/polyICLC Vaccine Promotes PDL1 Expression on CD163$^-$ Cells

Notwithstanding the limited number of treated patients, and despite the positive changes in the tumor microenvironment, the priming of an immune response against a tumor associated antigen, and the removal of all the tumor mass by salvage surgery, we did not observe a dramatic reduction of tumor recurrence in this high risk population (**Supplementary Figure 6**). We thus evaluated whether the higher number of activated T cells in the tumor may elicit additional mechanisms of immune escape. Indeed, in HNSCC as well as in other malignancies IFNγ released by CD8$^+$T cells was shown to upregulate PDL1 on neoplastic cells (57–59). We thus evaluated the expression of the checkpoint molecule PDL1 and the macrophage marker CD163 by image cytometry in the tumor of the enrolled patients. In the control untreated patients, PDL1 was mostly confined in CD163$^+$macrophage at the tumor edge whereas tumor and normal surrounding tissues expressed low level of this protein (**Figure 6A** and **Supplementary Figure 7**).

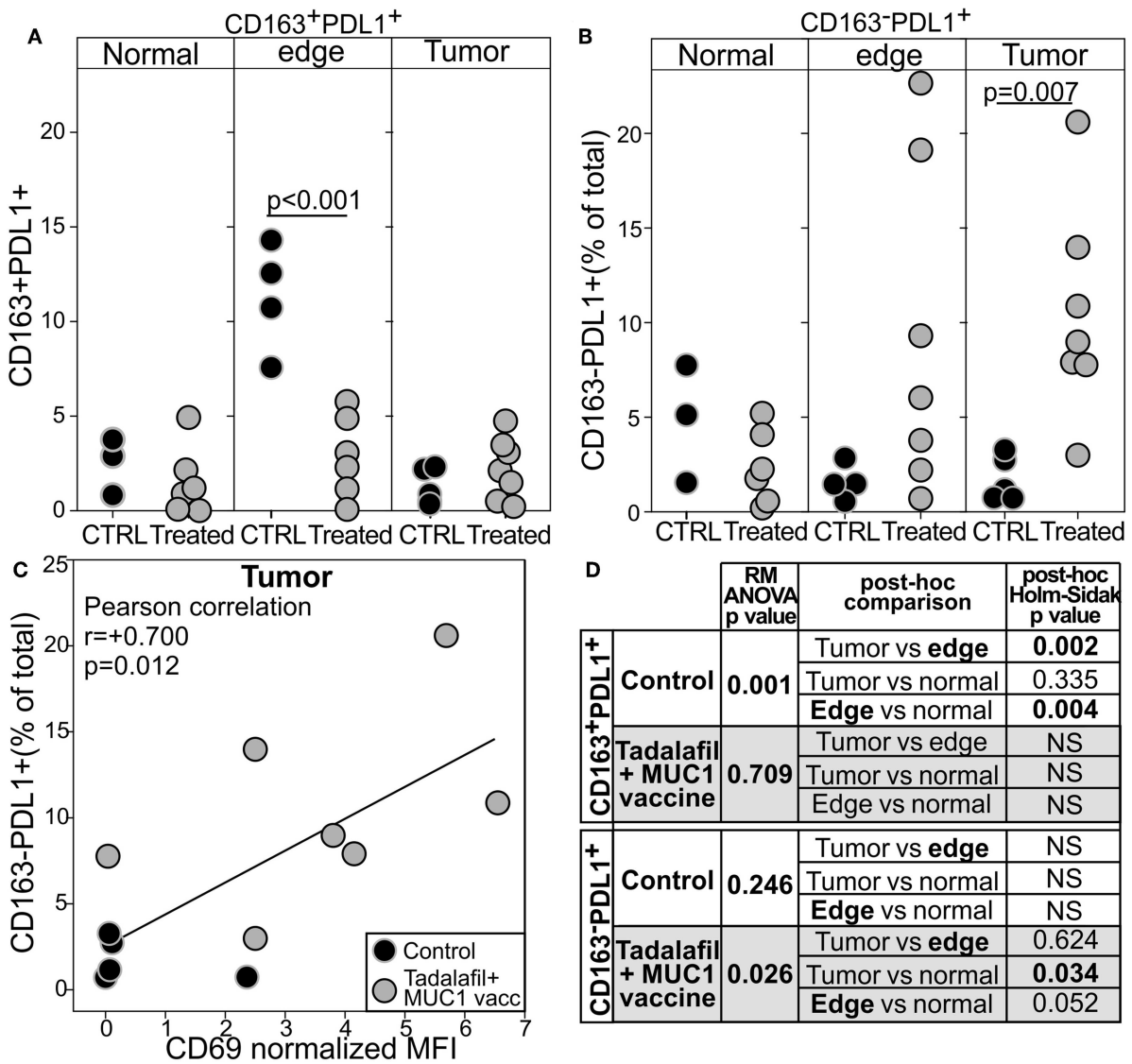

FIGURE 6 | Tadalafil and MUC1/polyICLC vaccine treatments modulate the expression of PDL1 in the tumor microenvironment. The expression of PDL1 within the CD163+ **(A)** or the CD163$^-$cells **(B)** was quantified by image cytometry in the tumor, at the tumor edge, or in "normal" surrounding tissue in the tumor specimen from the control (black filled circle) or Tadalafil and MUC1/polyICLC vaccine treated (gray filled circle) patients. Two way *T*-test *p*-value are reported. **(C)** Correlation between the expression of CD69 in the CD8$^+$T cells and PDL1 expression on the CD163$^-$cells. **(D)** Summary of the one way RM ANOVA analysis.

Conversely, in the treated patients CD163$^+$PDL1$^+$macrophages were significantly lower in the tumor edge and at concentration levels similar to that of the surrounding tissue (**Figure 6A** and **Supplementary Figure 7**). However, PDL1 expression at the tumor edge of the treated patients did not differ from that of the control patients because of an increase of this inhibitory marker in the CD163$^-$cells (**Figure 6B**). Indeed, the intratumoral expression of PDL1 was significantly higher in treated patients than in the control patients and confined mostly in the CD163$^-$ cells (**Figure 6B** and **Supplementary Figure 7**). Interestingly, the level of expression of PDL1 directly correlated with the expression of CD69 on the tumor infiltrating CD8$^+$cells, suggesting the instauration of a T cell dependent mechanism of PDL1 upregulation and immune escape (**Figure 6C**).

These data suggest that the beneficial activity of Tadalafil and MUC1 vaccine might be hindered by this corresponding upregulation of PDL1. Indeed, KMplotter analysis [KMplot.com, (60)] on RNAseq data from 499 patients with HNSCC reveals that the expression of CD8a and CD69 mRNA in the tumor well-correlate with improved survival (**Figure 7**). Addition of PDL1 (CD274) to the gene signature interrogated, drastically reduced the benefit of a higher infiltration in the tumor of CD8$^+$CD69$^+$ cells. No negative effects were noted when only expression of CD274 was evaluated.

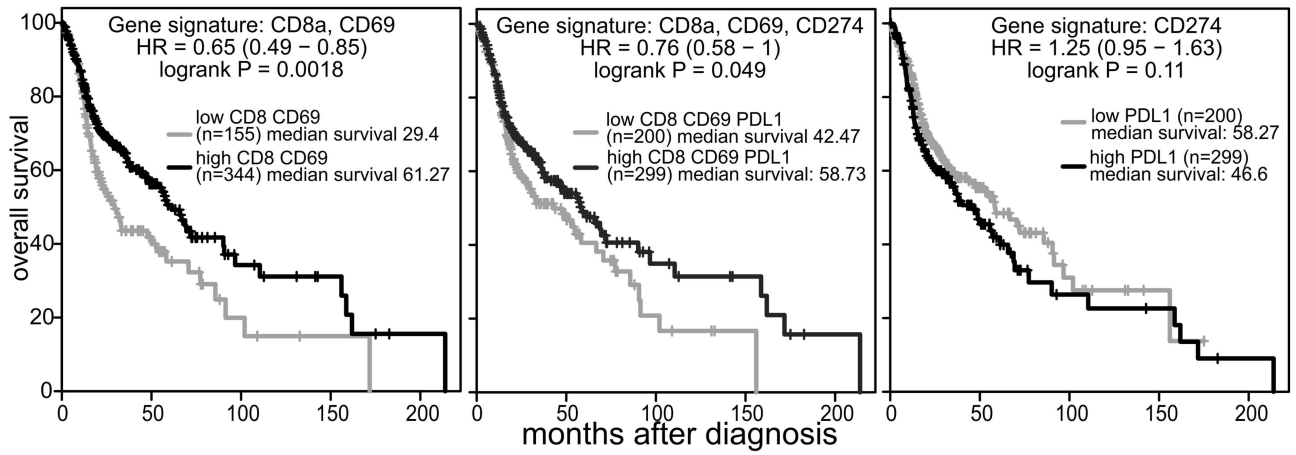

FIGURE 7 | PDL1 expression limits the beneficial prognostic value of CD8a and CD69 in the tumor. KM plotter analysis (kmplot.com) was performed on tumor RNAseq data from patients with HNSCC ($n = 499$, all tumor stages) using the mean expression of the indicated genes with weight =1 and auto select best cutoff selected.

DISCUSSION

This phase 1 clinical trial, designed in patients with recurrent HNSCC undergoing salvage surgery to evaluate the safety of combining chronic PDE5 inhibition to reverse tumor-induced immunosuppression, and MUC1/polyICLC immunization to prime a tumor specific immune response, seems to confirm the previous clinical evidence indicating beneficial actions for these two interventions in patients with malignancies. Indeed, initial case reports indicate an antitumor activity of PDE5 inhibition in patients with Waldenstrom's macroglobulinemia (61), B-cell chronic lymphocytic leukemia (62), and penile cancer (63). Tadalafil was successfully used to treat a patient with end-stage relapsed/refractory multiple myeloma (64), generating a dramatic and durable anti-myeloma immune response and clinical response with associated transfusion independence and improvement in quality of life (64).

Clinical trials are being performed in colorectal cancer (NCT02998736), Glioma (NCT01817751), abdominal malignancies (NCT02998736), advanced solid tumors (NCT02466802), pancreatic cancer (NCT01342224), myelodysplastic syndrome (NCT03259516), multiple myeloma (NCT01858558), metastatic melanoma (EudraCT-No: 2011-003273-28), and Head and Neck squamous cell carcinoma (NCT00843635, NCT00894413, NCT01697800, NCT03238365, NCT02544880). To date, data are available only for our two previous clinical trials in HNSCC and for a dose escalating open label clinical trial in progressive metastatic melanoma (65). In these trials, chronic tadalafil treatment lowered MDSCs and Treg in the blood and at the tumor site (32, 33, 65), restored the immune response to recall antigens (32), enhanced the expression of CD3 ζ-chain in $CD8^+$T cells (32), primed/enhanced the tumor specific immune response, and increased the number of tumor infiltrating T cells (33). However, notwithstanding the low number of patients enrolled in these trials, the positive immunomodulatory actions of PDE5 inhibition were associated with no clinical benefits (**Supplementary Figure 1**), although disease stabilization was reported for few patients in the melanoma trial and in case reports (64, 65).

Most of the beneficial immunomodulations of Tadalafil are confirmed in our ongoing phase 1 clinical Trial. Indeed, treatment was associated with a reduction of mMDSC and Treg in the blood and at the tumor site, an increase in the expression of CD3ζ chain at the tumor site and a higher infiltration of activated $CD8^+$ T cells at the tumor site (**Figures 3**, **5**).

After confirming MUC1 as a tumor specific antigen in recurrent HNSCC (**Figure 1**), for the first time we evaluated the safety and immunological potential of combining the MUC1 peptide/polyICLC vaccine with Tadalafil treatment. Despite finding a detectable IgG immune response in only 2 of the 6 treated patients (**Figure 4A**), the combined treatment seems to increase T cell reactivity to MUC1 (2–3% of T cell proliferation within the CD4 or CD8 gates to the relevant peptide) in most of the evaluable patients after 4 treatment courses (**Figure 4B**), and a significant correlation was found between the activation of tumor infiltrating lymphocytes and MUC1 expression in the tumor (**Figure 5**). Notwithstanding the low number of patients evaluated, taken together, these data suggest a possible priming of MUC1 immunity in most patients with $MUC1^+$tumors. Notwithstanding the low number of patients evaluated, taken together, these data suggest a possible priming of MUC1 immunity in most patients with $MUC1^+$tumors.

The combined treatment of Tadalafil and MUC1 vaccine was well-tolerated with no serious side effects, and no treatment limiting toxicity observed. One subject was withdrawn from the study for the development of an asymptomatic autoimmune disorder as determined by the detection of anti-nuclear antibodies while on treatment. Subsequent ANA testing in

this subject did revert to normal, with no clinical signs of autoimmunity detected at any time during treatment or after treatment discontinuation. The ANA test was selected as a screening tool for autoimmune disease for this trial, with a positive ANA test considered an exclusion criteria for enrollment. It should be noted that a total of 5 subjects otherwise eligible for enrollment in the phase I trial were excluded because of an asymptomatic positive ANA. While the study subject's development of a positive ANA while on treatment was interpreted as a potential sign of a treatment induced asymptomatic autoimmune disorder, and the subject was withdrawn from further treatment accordingly, this significant incidence of asymptomatic positive ANA in this patient cohort also raises the possibility that this finding may have been unrelated to the study intervention. Regardless, the combined immunologic interventions of this trial did not result in any clinically symptomatic autoimmune disease.

While this phase I study was not designed to demonstrate clinical efficacy of the study drug combination, the very poor prognosis and the expected high recurrence rate of the patient cohort studied provided for the potential identification of clinical efficacy should a dramatic clinical effect be demonstrated. Despite complete surgical extirpation of tumor and the addition of PDE5 inhibition and MUC1/PolyICLC vaccination, however, no such dramatic clinical benefit was detected (**Supplementary Figure 6**). This prompted us to evaluate whether additional mechanisms of immune escape were induced after reversal of immune exclusion.

Evaluation of PDL1 expression on macrophages and on CD163 negative cells at the tumor site via image cytometry suggest that while Tadalafil and/or polyICLC vaccine are effective in reducing $PDL1^{+}$macrophage at the tumor edge, the increase of $CD69^{+}$T cells within the tumor promotes (**Figure 5**) the expression of this inhibitory molecules on $CD163^{-}$cells (**Figure 6**). Indeed, a prominent role of activated T cells secreting type 2 interferon is emerging as inducer of PDL1 in neoplastic cells (57–59). For example, cisplatin and IFNγ have been shown to upregulate PDL1 on cell lines of HNSCC (66) and the secretion of this cytokine by activated CTL at the tumor site play a key role in the upregulation this checkpoint molecules in gastric cancer cells (67). In line with these observations we did find an intriguing correlation between the expression of CD69 in CTL at the tumor site and the expression of PDL1 (**Figure 6**).

It is important to note that our phase 1 study is limited by the low number of patients enrolled, by the open label single arm design among the treated patients, by the absence of randomization between the control and treated patients, and by the fact that the design of this phase 1 lead-in clinical trial does not allow for the discrimination between the immunological effects of Tadalafil and the immunological effects of the MUC1/polyICLC vaccine. Notwithstanding these limitations, however, to our knowledge this trial provides the first evidence that the combination of Tadalafil and the anti-MUC/polyICLC vaccine can reverse immune exclusion but also promote the upregulation of PDL1 as additional mechanisms of tumor escape. The notion that PDL1 upregulation may limit the efficacy of Tadalafil and vaccine based immunotherapy is further supported by the analysis of public RNAseq database. These analysis indicates that the beneficial prognostic role of the CD8CD69 signature in HNSCC is partially decreased by PDL1 expression.

Taken together, the interim analyses of this phase 1 clinical trial indicate that the treatment combination is safe and well-tolerated, can reverse immune exclusion, but can also promotes PDL1 upregulation. The latter finding provides a mechanism by which the proposed treatment combination may have offsetting immunologic outcomes. As such, a decision has been made to suspend accrual to the randomized phase II trial as designed given this potential limitation in experimental treatment efficacy. Instead, a new combinatorial intervention is being explored that conjugates salvage surgery, inhibition of PDE5, priming of an anti-tumor immune response, and checkpoint inhibition.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Protocol Review Committee of the Sylvester Comprehensive Cancer Center of the University of Miami Miller School of Medicine and as reviewed and approved under IND 16403 by the Food and Drug Administration, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of the University of Miami.

AUTHOR CONTRIBUTIONS

DW designed the study, oversaw the clinical trial and all the regulatory aspects, performed the salvage surgery, wrote the paper and interpreted the data. SZ performed the flow and image cytometry experiments and analyzed the data. IR provided the statistical support and analyzed the data. ZS and FC helped with patient enrollments and performed salvage surgery. MA helped with the patient follow up of our former clinical trial and with initial observation of PDL1 expression. CG-F provided the pathologic expertise. CR helped with blood processing and IHC analysis. PS designed the study, the immune monitoring assays, analyzed and interpreted the data and wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01206/full#supplementary-material

REFERENCES

1. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA Oncol.* (2017) 3:524–48. doi: 10.1001/jamaoncol.2016.5688
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin.* (2015) 65:5–29. doi: 10.3322/caac.21254
3. Janot F, de Raucourt D, Benhamou E, Ferron C, Dolivet G, Bensadoun RJ, et al. Randomized trial of postoperative reirradiation combined with chemotherapy after salvage surgery compared with salvage surgery alone in head and neck carcinoma. *J Clin Oncol.* (2008) 26:5518–23. doi: 10.1200/jco.2007.15.0102
4. Goodwin WJ Jr. Salvage surgery for patients with recurrent squamous cell carcinoma of the upper aerodigestive tract: when do the ends justify the means? *Laryngoscope.* (2000) 110(3 Pt 2 Suppl. 93):1–18. doi: 10.1097/00005537-200003001-00001
5. Linehan D, Noel MS, Hezel AF, Wang-Gillam A, Eskens F, Sleijfer S, et al. Overall survival in a trial of orally administered CCR2 inhibitor CCX872 in locally advanced/metastatic pancreatic cancer: Correlation with blood monocyte counts. *J Clinl Oncol.* (2018) 36(5 Suppl.):92. doi: 10.1200/JCO.2018.36.5_suppl.92
6. Shih K, Arkenau HT, Infante JR. Clinical impact of checkpoint inhibitors as novel cancer therapies. *Drugs.* (2014) 74:1993–2013. doi: 10.1007/s40265-014-0305-6
7. Rothschild U, Muller L, Lechner A, Schlosser HA, Beutner D, Laubli H, et al. Immunotherapy in head and neck cancer - scientific rationale, current treatment options and future directions. *Swiss Med Wkly.* (2018) 148:w14625. doi: 10.4414/smw.2018.14625
8. Bauml J, Seiwert TY, Pfister DG, Worden F, Liu SV, Gilbert J, et al. Pembrolizumab for platinum- and cetuximab-refractory head and neck cancer: results from a single-arm, phase II study. *J Clin Oncol.* (2017) 35:1542–9. doi: 10.1200/jco.2016.70.1524
9. Ferris RL, Blumenschein GR, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Two-year update from checkmate 141: outcomes with nivolumab (Nivo) vs investigator's choice (IC) in recurrent or metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN) in the overall population and PD-L1 subgroups. *Int J Rad Oncol Biol Phys.* (2018) 100:1317. doi: 10.1016/j.ijrobp.2017.12.300
10. Saleh K, Eid R, Haddad FG, Khalife-Saleh N, Kourie HR. New developments in the management of head and neck cancer - impact of pembrolizumab. *Therapeut Clin Risk Manag.* (2018) 14:295–303. doi: 10.2147/TCRM.S125059
11. Zuur CL, Elbers JBW, van der Leun A, van den Brekel M, Tan B, Jasperse B, et al. Immunomodulation by the combination of ipilimumab and nivolumab neoadjuvant to (salvage) surgery in advanced or recurrent head and neck carcinoma, IMCISION, an investigator-initiated phase-Ib/II trial (N16IMC, NCT03003637). *J Clin Oncol.* (2018) 36(15 Suppl.):e18020. doi: 10.1200/JCO.2018.36.15_suppl.e18020
12. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science.* (2015) 348:74–80. doi: 10.1126/science.aaa6204
13. Maleki Vareki S. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. *J ImmunoTher Cancer.* (2018) 6:157. doi: 10.1186/s40425-018-0479-7
14. Hoesli R, Birkeland AC, Rosko AJ, Issa M, Chow KL, Michmerhuizen NL, et al. Proportion of CD4 and CD8 tumor infiltrating lymphocytes predicts survival in persistent/recurrent laryngeal squamous cell carcinoma. *Oral oncology.* (2018) 77:83–9. doi: 10.1016/j.oraloncology.2017.12.003
15. Lu C, Redd PS, Lee JR, Savage N, Liu K. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. *Oncoimmunology.* (2016) 5:e1247135–e1247135. doi: 10.1080/2162402X.2016.1247135
16. Moschetta M, Uccello M, Kasenda B, Mak G, McClelland A, Boussios S, et al. Dynamics of neutrophils-to-lymphocyte ratio predict outcomes of PD-1/PD-L1 blockade. *BioMed Res Int.* (2017) 2017:5. doi: 10.1155/2017/1506824
17. Ameratunga M, Chénard-Poirier M, Moreno Candilejo I, Pedregal M, Lui A, Dolling D, et al. Neutrophil-lymphocyte ratio kinetics in patients with advanced solid tumours on phase I trials of PD-1/PD-L1 inhibitors. *Eur J Cancer.* (2018) 89:56–63. doi: 10.1016/j.ejca.2017.11.012
18. Chen M-F, Tsai M-S, Chen W-C, Chen P-T. Predictive value of the pretreatment neutrophil-to-lymphocyte ratio in head and neck squamous cell carcinoma. *J Clin Med.* (2018) 7:294. doi: 10.3390/jcm7100294
19. Parikh K, Kumar A, Ahmed J, Anwar A, Puccio C, Chun H, et al. Peripheral monocytes and neutrophils predict response to immune checkpoint inhibitors in patients with metastatic non-small cell lung cancer. *Cancer Immunol Immunother.* (2018) 67:1365–70. doi: 10.1007/s00262-018-2192-2
20. Parikh K, Kumar A, Ahmed J, Anwar A, Puccio C, Chun HG, et al. Monocytes and neutrophils as a predictive marker of response to immune checkpoint inhibitors (ICI) in metastatic non-small cell lung cancer (mNSCLC). *J Clin Oncol.* (2018) 36(15 Suppl.): e21165. doi: 10.1200/JCO.2018.36.15_suppl.e21165
21. Sacdalan DB, Lucero JA, Sacdalan DL. Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: a review and meta-analysis. *OncoTargets Ther.* (2018) 11:955–65. doi: 10.2147/OTT.S153290
22. Kim HR, Park SM, Seo SU, Jung I, Yoon HI, Gabrilovich DI, et al. The ratio of peripheral regulatory T cells to Lox-1(+) polymorphonuclear myeloid-derived suppressor cells predicts the early response to Anti-PD-1 therapy in patients with non-small cell lung cancer. *Am J Respir Crit Care Med.* (2019) 199:243–6. doi: 10.1164/rccm.201808-1502LE
23. Serafini P. Myeloid derived suppressor cells in physiological and pathological conditions: the good, the bad, and the ugly. *Immunol Res.* (2013) 57:172–84. doi: 10.1007/s12026-013-8455-2
24. Zilio S, Serafini P. Neutrophils and granulocytic MDSC: the janus god of cancer immunotherapy. *Vaccines.* (2016) 4:31. doi: 10.3390/vaccines4030031
25. Davis RJ, Moore EC, Clavijo PE, Friedman J, Cash H, Chen Z, et al. Anti-PD-L1 efficacy can be enhanced by inhibition of myeloid-derived suppressor cells with a selective inhibitor of PI3Kdelta/gamma. *Cancer Res.* (2017) 77:2607–19. doi: 10.1158/0008-5472.Can-16-2534
26. Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W, et al. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J Exp Med.* (2006) 203:2691–702. doi: 10.1084/jem.20061104
27. Serafini P, Mgebroff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res.* (2008) 68:5439–49. doi: 10.1158/0008-5472.CAN-07-6621

28. Capuano G, Rigamonti N, Grioni M, Freschi M, Bellone M. Modulators of arginine metabolism support cancer immunosurveillance. *BMC Immunol.* (2009) 10:1–1. doi: 10.1186/1471-2172-10-1
29. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W, et al. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. *Proc Natl Acad Sci USA.* (2011) 108:17111–6. doi: 10.1073/pnas.1108121108
30. Rigamonti N, Capuano G, Ricupito A, Jachetti E, Grioni M, Generoso L, et al. Modulators of arginine metabolism do not impact on peripheral T-cell tolerance and disease progression in a model of spontaneous prostate cancer. *Clin Cancer Res.* (2011) 17:1012–23. doi: 10.1158/1078-0432.Ccr-10-2547
31. Confino H, Schmidt M, Efrati M, Hochman I, Umansky V, Kelson I, et al. Inhibition of mouse breast adenocarcinoma growth by ablation with intratumoral alpha-irradiation combined with inhibitors of immunosuppression and CpG. *Cancer Immunol Immunother.* (2016) 65:1149–58. doi: 10.1007/s00262-016-1878-6
32. Califano JA, Khan Z, Noonan KA, Rudraraju L, Zhang Z, Wang H, et al. Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:30–8. doi: 10.1158/1078-0432.ccr-14-1716
33. Weed DT, Vella JL, Reis IM, De la Fuente AC, Gomez C, Sargi Z, et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res.* (2015) 21:39–48. doi: 10.1158/1078-0432.ccr-14-1711
34. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res.* (2009) 15:5323–37. doi: 10.1158/1078-0432.CCR-09-0737
35. Rubin JS, Bloor BK, Hart IR, Morgan PR. muc-1 gene expression in head and neck squamous cell carcinomas. *J Laryngol Otol.* (2000) 114:772–6. doi: 10.1258/0022215001904121
36. Croce MV, Rabassa ME, Price MR, Segal-Eiras A. MUC1 mucin and carbohydrate associated antigens as tumor markers in head and neck squamous cell carcinoma. *Pathol Oncol Res.* (2001) 7:284–91. doi: 10.1053.paor.2001.0338
37. Croce MV, Rabassa ME, Pereyra A, Segal-Eiras A. Influence of sialic acid removal on MUC1 antigenic reactivity in head and neck carcinoma. *Pathol Oncol Res.* (2005) 11:74–81. doi: 10.1007/BF02893370
38. Young MR, Neville BW, Chi AC, Lathers DM, Boyd Gillespie M, Day TA. Oral premalignant lesions induce immune reactivity to both premalignant oral lesions and head and neck squamous cell carcinoma. *Cancer Immunol Immunother.* (2007) 56:1077–86. doi: 10.1007/s00262-006-0242-7
39. Croce MV, Rabassa ME, Pereyra A, Segal-Eiras A. Differential expression of MUC1 and carbohydrate antigens in primary and secondary head and neck squamous cell carcinoma. *Head Neck.* (2008) 30:647–57. doi: 10.1002/hed.20756
40. Hamada T, Nomura M, Kamikawa Y, Yamada N, Batra SK, Yonezawa S, et al. DF3 epitope expression on MUC1 mucin is associated with tumor aggressiveness, subsequent lymph node metastasis, and poor prognosis in patients with oral squamous cell carcinoma. *Cancer.* (2012) 118:5251–64. doi: 10.1002/cncr.27542
41. Lu H, Liang D, Zhu Y, Xu W, Zhou K, Liu L, et al. Prognostic and clinicopathological significance of MUC expression in head and neck cancer: a systematic review and meta-analysis. *Oncotarget.* (2017) 8:96359–72. doi: 10.18632/oncotarget.19648
42. Beckhove P, Warta R, Lemke B, Stoycheva D, Momburg F, Schnölzer M, et al. Rapid T cell–based identification of human tumor tissue antigens by automated two-dimensional protein fractionation. *J Clin Investig.* (2010) 120:2230–42. doi: 10.1172/JCI37646
43. Kovjazin R, Volovitz I, Kundel Y, Rosenbaum E, Medalia G, Horn G, et al. ImMucin: a novel therapeutic vaccine with promiscuous MHC binding for the treatment of MUC1-expressing tumors. *Vaccine.* (2011) 29:4676–86. doi: 10.1016/j.vaccine.2011.04.103
44. Limacher JM, Quoix E. TG4010: A therapeutic vaccine against MUC1 expressing tumors. *Oncoimmunology.* (2012) 1:791–2. doi: 10.4161/onci.19863
45. Beatty PL, Finn OJ. Preventing cancer by targeting abnormally expressed self-antigens: MUC1 vaccines for prevention of epithelial adenocarcinomas. *Ann N Y Acad Sci.* (2013) 1284:52–6. doi: 10.1111/nyas.12108
46. Kimura T, Finn OJ. MUC1 immunotherapy is here to stay. *Expert Opin Biol Ther.* (2013) 13:35–49. doi: 10.1517/14712598.2012.725719
47. Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. *Cancer Prev Res.* (2013) 6:18–26. doi: 10.1158/1940-6207.CAPR-12-0275
48. Cascio S, Faylo JL, Sciurba JC, Xue J, Ranganathan S, Lohmueller JJ, et al. Abnormally glycosylated MUC1 establishes a positive feedback circuit of inflammatory cytokines, mediated by NF-kappaB p65 and EzH2, in colitis-associated cancer. *Oncotarget.* (2017) 8:105284–98. doi: 10.18632/oncotarget.22168
49. Elbers JBW, Al-Mamgani A, van den Brekel MWM, Józwiak K, de Boer JP, Lohuis, PJ, et al. Salvage surgery for recurrence after radiotherapy for squamous cell carcinoma of the head and neck. *Otolaryngol Head Neck Surgery.* (2018). doi: 10.1177/0194599818818443. [Epub ahead of print].
50. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol.* (2016) 1:8943. doi: 10.1126/sciimmunol.aaf8943
51. Reichert TE, Day R, Wagner EM, Whiteside TL. Absent or low expression of the zeta chain in T cells at the tumor site correlates with poor survival in patients with oral carcinoma. *Cancer Res.* (1998) 58:5344–7.
52. Kuss I, Saito T, Johnson JT, Whiteside TL. Clinical Significance of Decreased ζ Chain Expression in Peripheral Blood Lymphocytes of Patients with Head and Neck Cancer. *Clinical Cancer Res.* (1999) 5:329–34.
53. Rodríguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *Immunol Rev.* (2008) 222:180–91. doi: 10.1111/j.1600-065X.2008.00608.x
54. Kanekiyo S, Hazama S, Takenouchi H, Nakajima M, Shindo Y, Matsui H, et al. IgG response to MHC class I epitope peptides is a quantitative predictive biomarker in the early course of treatment of colorectal cancer using therapeutic peptides. *Oncol Rep.* (2018) 39:2385–92. doi: 10.3892/or.2018.6288
55. Weed DT, Walker G, De La Fuente AC, Nazarian R, Vella JL, Gomez-Fernandez CR, et al. FOXP3 subcellular localization predicts recurrence in oral squamous cell carcinoma. *PLoS ONE.* (2013) 8:e71908. doi: 10.1371/journal.pone.0071908
56. Magg T, Mannert J, Ellwart JW, Schmid I, Albert MH. Subcellular localization of FOXP3 in human regulatory and nonregulatory T cells. *Eur J Immunol.* (2012) 42:1627–38. doi: 10.1002/eji.201141838
57. Mandal R, Senbabaoglu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight.* (2016) 1:e89829. doi: 10.1172/jci.insight.89829
58. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest.* (2017) 127:2930–40. doi: 10.1172/jci91190
59. Lin W, Chen M, Hong L, Zhao H, Chen Q. Crosstalk between PD-1/PD-L1 blockade and its combinatorial therapies in tumor immune microenvironment: a focus on HNSCC. *Front Oncol.* (2018) 8:532. doi: 10.3389/fonc.2018.00532
60. Lanczky A, Nagy A, Bottai G, Munkacsy G, Szabo A, Santarpia L, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat.* (2016) 160:439–46. doi: 10.1007/s10549-016-4013-7
61. Treon SP, Tournilhac O, Branagan AR, Hunter Z, Xu L, Hatjiharissi E, et al. Clinical responses to sildenafil in Waldenstrom's macroglobulinemia. *Clin Lymphoma.* (2004) 5:205–7. doi: 10.3816/CLM.2004.n.029

62. Sarfati M, Mateo V, Baudet S, Rubio M, Fernandez C, Davi F, et al. Sildenafil and vardenafil, types 5 and 6 phosphodiesterase inhibitors, induce caspase-dependent apoptosis of B-chronic lymphocytic leukemia cells. *Blood*. (2003) 101:265. doi: 10.1182/blood-2002-01-0075
63. Huilgol NG, Jain A. A new indication of sildenafil in medicine: hypoxic cell sensitizer for penile cancer. *J Cancer Res Ther*. (2006) 2:132–5. doi: 10.4103/0973-1482.27589
64. Noonan KA, Ghosh N, Rudraraju L, Bui M, Borrello I. Targeting immune suppression with PDE5 inhibition in end-stage multiple myeloma. *Cancer Immunol Res*. (2014) 2:725–31. doi: 10.1158/2326-6066.Cir-13-0213
65. Hassel JC, Jiang H, Bender C, Winkler J, Sevko A, Shevchenko I, et al. Tadalafil has biologic activity in human melanoma. Results of a pilot trial with Tadalafil in patients with metastatic Melanoma (TaMe). *Oncoimmunology*. (2017) 6:e1326440. doi: 10.1080/2162402x.2017.1326440
66. Ock CY, Kim S, Keam B, Kim S, Ahn YO, Chung EJ, et al. Changes in programmed death-ligand 1 expression during cisplatin treatment in patients with head and neck squamous cell carcinoma. *Oncotarget*. (2017) 8:97920–7. doi: 10.18632/oncotarget.18542
67. Mimura K, Teh JL, Okayama H, Shiraishi K, Kua LF, Koh V, et al. PD-L1 expression is mainly regulated by interferon gamma associated with JAK-STAT pathway in gastric cancer. *Cancer Sci*. (2018) 109:43–53. doi: 10.1111/cas.13424

Conflict of Interest Statement: PS is named as inventor in patent owned by Johns Hopkins University regarding the use of PDE5 inhibitors as immune modulator.

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Myeloid Derived Suppressor Cells: Key Drivers of Immunosuppression in Ovarian Cancer

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The presence of tumor infiltrating lymphocytes (TILs) is associated with a longer overall survival in advanced stage epithelial ovarian cancer. Despite the prognostic impact of TILs, response to checkpoint-inhibitors and antigen-specific active immunotherapy is limited in ovarian cancer. The goal of our study was to investigate the interaction between ovarian cancer and the innate and adaptive immune system in the ID8-fLuc syngeneic ovarian cancer mouse model. For the *in vivo* experiments C57BL/6, B6.129S7-Rag1^{tm1Mom}/J, and B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice were inoculated with ID8-fLuc. *In vivo* depletion experiments were performed using clodronate liposomes (CL), anti-CD8a, anti-GR1, anti-colony stimulating factor 1 (anti-CSF1), and TMβ1 (anti-CD122). Immune read out was performed by fluorescent activated cell sorting analysis for effector T cells, regulatory T cells, natural killer cells, B cells, macrophages, and myeloid derived suppressor cells (MDSC), immunohistochemistry for MDSC and tumor-associated macrophages (TAM) and immunofluorescence for M1 and M2 TAM in the vascular context. The effect of MDSC on T cell proliferation and phenotype were studied *in vitro*. We discovered that the absence of T and B cells did not influence tumor growth or survival of B6.129S7-Rag1^{tm1Mom}/J mice compared to immunocompetent C57BL/6 mice. CL-induced macrophage depletion promoted tumor proliferation and shortened survival in C57BL/6 mice ($p = 0.004$) and in B6.129S7-Rag1^{tm1Mom}/J mice ($p = 0.0005$). During CL treatment, we observed a clear increase of pro-inflammatory cytokines ($p \leq 0.02$) and monocytic MDSC ($p \leq 0.01$). Selective depletion of MDSC by anti-GR1 improved survival, certainly in comparison to mice treated with anti-CSF1 ($p = 0.01$—median survival 91 vs. 67.5 days). B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice displayed to a longer median survival compared to C57BL/6 mice (90 vs. 76 days). MDSC activated by ID8-fLuc conditioned medium or ascites of tumor-bearing mice showed T

cell suppressive functions *in vitro.* Based on these findings, we conclude that the adaptive immune system does not efficiently control tumor growth in the ID8-fLuc model. In addition, we discovered a prominent role for MDSC as the driver of immunosuppression in the ID8-fLuc ovarian cancer mouse model.

Keywords: ovarian cancer, immunosuppression, myeloid derived suppressor cells, adaptive immune system, innate immune system

INTRODUCTION

Ovarian cancer is the 5th leading cause of cancer death for women in developed countries (1). Standard treatment for advanced stage epithelial ovarian cancer is cytoreductive surgery in combination with platin-based chemotherapy (2). Despite radical surgery and excellent responses to first line chemotherapy, most patients diagnosed with advanced ovarian cancer do not survive beyond 5 years after diagnosis because of treatment-resistant recurrences (3). Ovarian cancer can be subdivided into four subtypes based on mRNA and miRNA expression, DNA copy number, DNA promotor methylation, and whole-exome DNA sequence analysis: immunoreactive, differentiated, proliferative, and mesenchymal (4). The immunoreactive subtype, which characterized by increased expression of CXCL11, CXCL10, and CXCR3, and displays the most favorable overall survival (OS) compared to the other subgroups (5). In line with this evidence, Zhang et al. demonstrated that the presence of tumor infiltrating lymphocytes (TILs) significantly correlates with improved survival in advanced epithelial ovarian cancer (6). In 2015, the Ovarian Cancer Action meeting suggested to study the interaction between ovarian cancer and the immune system, in order to develop strategies aimed at potentiating the anti-tumor immune response (7). Despite these efforts, only a limited number of ovarian cancer patients have responded to checkpoint-inhibitor therapy (8–10). In addition to this, no significant survival benefit was observed in ovarian cancer patients receiving antigen-specific active immunotherapy to date, most likely due to an overwhelming immunosuppression (11, 12).

Unlike the adaptive immune system, the innate immune system has not been extensively studied in the context of ovarian cancer, where it might be a key driver of immunosuppression. In previous studies, a high number of alternatively activated M2 tumor-associated macrophages (TAM) in ascites has been linked to poor clinical outcome. Furthermore, given the positive effects of anti- vascular endothelial growth factor (VEGF) treatment in ovarian cancer and the evidence that TAM are an important source of VEGF, targeting TAMs could also be interesting therapeutic option in this context (13–15). In addition, Cui et al. demonstrated that a high number of $CD33^+$ cells in the tumor microenvironment was prognostic for shorter PFS ($p = 0.006$) and OS ($p = 0.02$) (16). The role of other innate immune cells, such as natural killer (NK) cells, dendritic cells, etc., remains unclear in ovarian cancer.

In this study, we discovered that depleting immune effector cells of the adaptive immune system ($CD8^+$ T cells) does not increase tumor growth or influence survival in the ID8-fLuc model. We therefore explored the role of the innate immune system in the inhibition of the adaptive immune response. We observed a key role for (monocytic) myeloid derived-suppressor cells (mMDSC) in immune surveillance in the ID8-fLuc model.

MATERIALS AND METHODS

Mice

Six- to eight-week-old mice were used. C57BL/6 and C57BL/6/BrDCHsd-Tyrc mice were obtained from Harlan/Envigo (Horst, Netherlands) or from an internal colony at KU Leuven. C57BL/6J-Tyrc-2J/J, B6.129S7-Rag1^{tm1Mom}/J, and B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice were obtained via Charles River from The Jackson Laboratory (Bar Harbor, ME, USA). For the *in vivo* experiment, only female mice were used. C57BL/6/BrDCHsd-Tyrc and C57BL/6J-Tyrc-2J/J are albino C57BL/6 mice, lacking all pigment from skin, hair and eyes.

B6.129S7-Rag1^{tm1Mom}/J are immune deficient mice with a C57BL/6 background, lacking for mature T or B cells (17). B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J are C57BL/6 mice that have a defect in the Myd88 cytosolic adapter, a protein which plays a central role in dendritic cells metabolism and in the immunosuppressive function of MDSC by activating NADPH oxidase and arginase-1 (18, 19).

Ovarian cancer was induced in the mice by intraperitoneal (IP) administration of 5×10^6 ID8-fLuc cells dissolved in 100 μL cold Phosphate-Buffered Saline (PBS). The ID8-fLuc cell line was transducted by the Laboratory of Molecular Virology and Gene Therapy and Leuven Viral Vector Core in our institute. All *in vivo* experiments were performed with 5–6 mice per group and passages 2–4 of the ID8-fLuc cells. No systematic mycoplasma testing was performed. Severely ill animals were euthanized following humane endpoints as previously described by our group (20). All animals were housed and treated according to the Federation for Laboratory Animal Science Associations guidelines (21). Ethical approval was obtained from the local Ethical Committee (p075/2014 and p125/2017).

Bioluminescence Imaging (BLI)

Non-invasive bioluminescence imaging (BLI) was used to evaluate tumor burden in albino C57BL/6/BrDCHsd-Tyrc and C57BL/6J-Tyrc-2J/J mice. As read-out, we used the maximum luminescence after administration of D-Luciferin (Promega, Madison, WI, USA) as a measure of viable tumor load. Image analysis was performed on the IVIS Spectrum Preclinical *in vivo* Imaging System (PerkinElmer, Waltham, MA, USA) at the Molecular Small Animal Imaging Centre (moSAIC) at the KU Leuven (22). The first scan was performed 1 week after tumor

challenge in order to obtain a baseline of tumor engraftment. Subsequent measurements were performed once a week until 6 weeks after inoculation. In the CD8 T cell depletion experiment mice were scanned only scanned twice (week 1 and week 6 after tumor inoculation).

In vivo Depletion Experiments

Clodronate Liposomes (CL) were purchased from Liposoma (Amsterdam, The Netherlands). We started treating the mice 1 week after tumor challenge with CL IP twice a week at a dosage of 0.05 mg/g bodyweight. As a control, PBS liposomes were used in preliminary experiments.

Depletion of $CD8^+$ T cells was achieved using anti-CD8a (clone 53-6.72) purchased from BioXCell (West Lebanon, NH, USA). Three weeks after tumor inoculation, we administered a loading dose of 0.5 mg per mouse IP on 3 consecutive days after which we performed weekly maintenance IP injections of 1 mg in accordance to manufacturers' protocol.

For the depletion of $NKp46^+$ NK cells we used TMβ1 (anti-CD122 monoclonal antibody), which was a kind gift of Ben Sprangers and Mark Waer (Lab of experimental transplantation, KU Leuven, Belgium). TMβ1 was produced in house by using the hybridoma technique. TMβ1 was administered IP at a dosage of 1 mg per mouse starting 1 day before tumor inoculation and continued at the same dosage twice a week.

Depletion of MDSC was achieved using anti-GR1 (Clone:RB6-8C5) purchased from BioXCell (West Lebanon, NH, USA). The monoclonal antibody was administered IP, at a dose of 10 mg/kg body weight, 3 times per week starting 1 week after inoculation.

A monoclonal antibody targeting colony stimulating factor 1 (CSF-1) (Clone:5A1) was used for the selective depletion of macrophages. Both the depleting antibody and the control antibody were bought from BioXCell (West Lebanon, NH, USA) and were administered IP. After a loading dose of 1 mg per mouse at day 21 after tumor challenge, a maintenance dose of 0.5 mg of anti-CSF1 or control antibody was administered once every 6 days IP.

Immunohistochemistry (IHC)

Tumor tissue from metastatic disease was stained for the presence of Ly6C. In brief, paraffin-embedded tissue slices were deparaffinized and rehydrated using graded ethanol. Endogenous peroxidase activity was blocked by 0.5% H_2O_2 in methanol. After washing, heat-mediated antigen retrieval was carried out at 37°C in hydrogen chloride buffer containing pepsine 0.04% during 10 min. After cooling down and washing, non-specific binding was blocked and sections were incubated overnight at 4°C with rat anti-mouse Ly6C primary antibody (1:200 dilution; Thermo Fisher, Merelbeke, Belgium). After washing, sections were incubated during 30 min with goat anti-rat biotinylated secondary antibody (dilution 1:100; Abcam, Cambridge, UK), followed by another 30 min with streptavidin/peroxidase (dilution 1:1,000; DAKO/Agilent, Haasrode, Belgium). Staining was performed using 3,3′-diaminobenzidine (DAB) during 10 min. Sections were counterstained with Mayer's Hematoxylin solution, dehydrated with ethanol and mounted in DePex medium. Images were acquired on Zeiss Axio Scan.Z1 using a x20 objective and ZEN2 software (Zeiss). Four random fields at 20x magnification were chosen and used to manually count positive cells. The mean of the four values was used for downstream analyses. IHC was scored by AVK using Image J software [National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin)].

Immunofluorescence Staining

Mice were sacrificed 33 days after tumor inoculation and peritoneal biopsies were taken. Tumor biopsies were prepared as 200 μm-thick vibratome sections, blocked and permeabilized in TNBT buffer [0.1 M Tris pH 7.4; NaCl 150 mM 0.5% blocking reagent from Perkin Elmer (Waltham, Massachusetts, USA), 0.5% Triton X-100] for 4 h at room temperature. Tissues were incubated overnight at 4°C with the following primary antibodies diluted in TNBT buffer: anti-glucose transporter-1 (Glut1) (Millipore, Burlington, Massachusetts, USA; 1:200 dilution), anti-Glut1 (Abcam, Cambridge, UK; 1:200 dilution), anti-major histocompatibility complex II (MHC-II) (Thermo Scientific, Waltham, Massachusetts, USA; 1:100 dilution) or anti-mannose receptor C type 1 (MRC1) (R&D Systems, Minneapolis, Minnesota, USA; 2 ug/ml). Next, slides were washed in TNBT buffer and incubated overnight at 4°C with the appropriate secondary antibody coupled with Alexa 488/555 (Life Technologies, Carlsbad, California, USA; 1:200 dilution) diluted in TNB Triton buffer. Tissues were washed and mounted on slides in fluorescent mounting medium (Dako/Agilent, Santa Clara, California, USA). Images were acquired using a Leica TCS SP8 confocal microscope. Semi-automated quantification analyses were performed using Fiji software (23).

Immune Monitoring

The immune status of mice was evaluated at predefined time points, as described in the specific experimental set-ups. Mice were anesthetized with 80 μL ketamine [100 mg/mL; Nimatek (Eurovet, Bladel, Nederland)] and blood was collected from the retro-orbital plexus using glass capillaries. Blood was centrifuged at 8,000 rcf for 10 min. Serum was collected and stored at −80°C for further analysis. Next, the animals were euthanized by cervical dislocation. Peritoneal washing with 10 mL of PBS was performed to collect the circulating immune cells in ascites and from the peritoneal lining. Peritoneal washings were centrifuged for 5 min at 500 rcf and resuspended. Supernatant was collected and stored at −80°C for cytokine analysis. Using a Lymphoprep (Stemcell technologies, Vancouver, Canda) gradient, immune cells were isolated from the cell suspension and analyzed with flow cytometry (FACS).

Using flow cytometry, dead cells were excluded via eFluor780 fixable viability dye staining (Affymetrix Inc. San Diego, CA, USA). Immune cells were stained for myeloid cells, T cells and B cells using antibody panels, which are available as **Supplementary Material** (**Supplementary Tables 1–3**, respectively). For the myeloid panel, the cells were permeabilized using Leucoperm (Bio-Rad Laboratories Inc., Kidlington, UK) in accordance to manufacturers' protocol and stained for CD206. Permeabilization in the T cell panel was achieved using the

eBioscience Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher scientific, Waltham, Massachusetts, USA) and cells were then stained for FoxP3. Samples were acquired on the BD LSRFortessa (BD Biosciences, San Jose, CA, USA) and the analysis was performed using FlowJo Analysis software (Flow Jo, LLC, Ashland, Oregon, USA).

Cytokines in serum and ascites were determined using cytometric bead assay technique (BD Biosciences, San Jose, CA, USA). Both serum and peritoneal washings/ascites were used undiluted. The analysis was performed in accordance to the manufacturers' protocol using flex sets for IL-1 β, GM-CSF, IL-6, IL-10, MIP-1α, MIP-1β, TNFα, and IFNγ. Samples acquisition was performed on the BD LSRFortessa (BD Biosciences, San Jose, CA, USA) and the analysis was performed using FCAP Array Software v3.0 (BD Biosciences, San Jose, CA, USA).

In vitro Experiments

MDSC were derived from bone marrow progenitor cells and splenocytes of C57BL/6 mice. Bone marrow progenitors, cells were isolated from bone marrow by flushing the long bones with PBS. For splenocytes, a single cell suspension was generated by passaging spleens through a 70 μm nylon strainer. From both splenocytes and bone marrow cells, dead cells were removed by the dead cell removal kit (130-090-101, Miltenyi Biotec, Bergisch Gladbach, Germany) in accordance to manufacturers' protocol. Next, MDSC were selected with the MDSC cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), which provides two fractions based on relative GR1 expression: the MDSC-DIM corresponding to mMDSC and the MDSC-HIGH corresponding to gMDSC. For the T cell fraction, $CD8^+$ T cells were selected from a single cell suspension of splenocytes using the $CD8^+$ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). T cells were activated by CD3/CD28 coated beads and cultured in medium supplemented with recombinant interleukin-2 (IL-2). Purity of all isolated cell types was verified by FACS.

In the first *in vitro* experiment naïve MDSC were exposed to ID8-fLuc conditioned medium. For this purpose, ID8-fLuc cells were grown in 96-well plates with trans well inserts (CoStar, Washington, D.C., USA), while MDSC were cultured in the inserts. Next the activated MDSC were co-cultured with $CD8^+$ T cells. We evaluated the proliferation of T cells by quantification of the CFSE (Affymetrix Inc. San Diego, CA, USA) dilution.

In the second experiment MDSC were cultured in the presence of supernatant derived from ascites of tumor bearing mice to investigate the role of soluble factors in ascites. Subsequently, the stimulated MDSC were co-cultured with activated T cells and stained for FACS using the staining panel in **Supplementary Table 4**. Dead cells were excluded from the analysis by use of the eFluor780 fixable viability dye (Affymetrix Inc. San Diego, CA, USA). Cells were acquired on the BD Canto-II (BD Biosciences, San Jose, CA, USA). Analysis was performed using FlowJo Analysis software (Flow Jo, LLC, Ashland, Oregon, USA).

Statistical Analysis

Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis and graphics. To evaluate statistical significance, α was set at 0.05. D'Agostino & Pearson omnibus

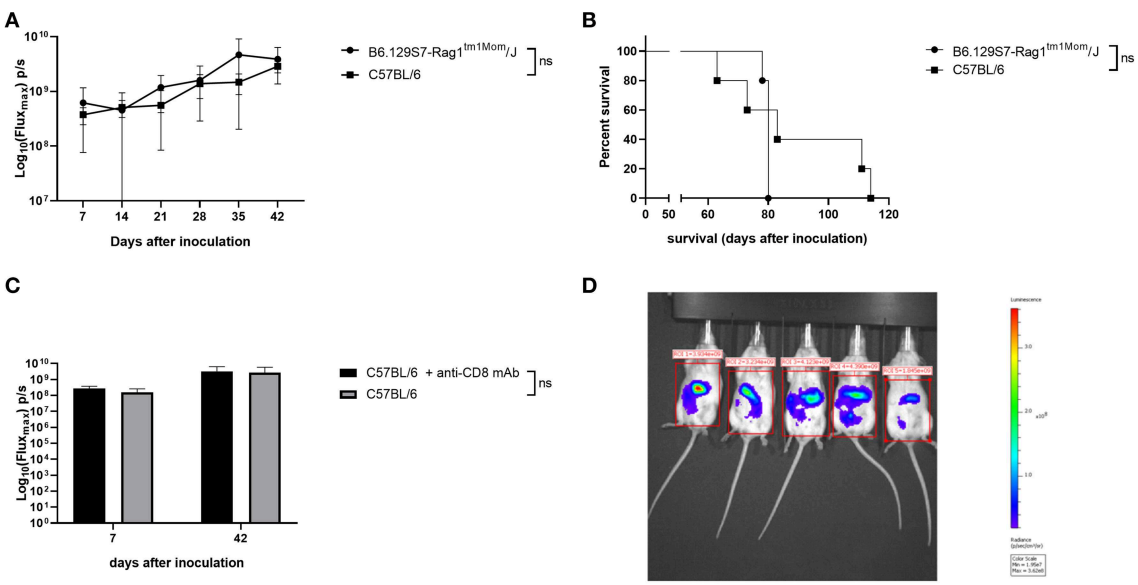

FIGURE 1 | Lack of cancer immune surveillance by the adaptive immune system in the ID8-fLuc model. **(A)** Evaluation of tumor growth using BLI, Log_{10} transformation of maximal flux in photons per second (p/s) are shown as mean with standard deviation. We observed no significant (ns) difference in tumor growth in the mice lacking mature T cells and B cells (B6.129S7-Rag1^{tm1Mom}/J) compared to the immunocompetent C57BL/6 mice. ($n = 5$ mice per group). **(B)** Kaplan-Meier curve showing the survival of B6.129S7-Rag1^{tm1Mom}/J mice compared to immunocompetent C57BL/6 mice. Median survival is 80 days for B6.129S7-Rag1tm1Mom/J and 83 days for C57BL/6 ($n = 5$ mice per group). **(C)** Follow-up of tumor growth using BLI in wild type mice that received CD8 depletion (C57BL/6 + anti-CD8a mAb) compared to untreated mice (C57BL/6). Imaging was performed 1 and 6 weeks after tumor inoculation. No significant differences between the groups were observed. ($n = 6$ mice per group). **(D)** Representative picture of BLI imaging taken with the IVIS Spectrum Preclinical *in vivo* Imaging System.

normality test was used to evaluate normality. performed. For continuous variables, data are presented as mean ± SD or medians (interquartile ranges) as appropriate. Between-group comparisons used the Mann–Whitney *U*-test or *t*-test depending on the sample size for continuous variables. In cases more than two groups are compared one-way ANOVA test was performed, followed by Turkey's multiple comparison test if $p < 0.05$. Log-rank testing was performed to compare survival curves.

RESULTS

Adaptive Immune Tolerance

We compared tumor growth of ID8-fLuc cells in B6.129S7-Rag1^{tm1Mom}/J mice to tumor growth in C57BL/6 mice using BLI. As shown in **Figure 1A**, there was no significant difference in tumor burden between immunocompetent mice (C57BL/6) and mice lacking mature T and B cells (B6.129S7-Rag1^{tm1Mom}/J). The B6.129S7-Rag1tm1Mom/J mice developed ascites at approximately the same moment as the C57BL/6 mice. There was no significant difference in survival between the two groups (**Figure 1B**). To investigate the specific role of CD8$^+$ T cells in immune surveillance in the ID8-fLuc ovarian cancer model, we performed a depletion experiment by which we inoculated C57BL/6 mice with ID8-fLuc and started treating the mice with anti-CD8 20 days after tumor inoculation (onset of exponential tumor growth phase, as demonstrated earlier) (20) In this experiment, we did not observe a difference in tumor burden 6 weeks after inoculation between anti-CD8 treated and control mice, which corresponds to the results obtained with B6.129S7-Rag1^{tm1Mom}/J mice (**Figures 1C,D**). Based on these findings, we can conclude that in the ID8-fLuc model the adaptive immune system has developed a tolerance against the tumor since knock-out or depletion of the adaptive immune system does not significantly influence tumor growth or survival. We therefore hypothesize that the innate immune system could play a role in rendering the effector cells of the adaptive immune system unfit for cancer immune surveillance in our model.

Influence of Macrophages on Tumor Growth and Survival the ID8-fLuc Model

In order to target innate immunosuppression, we treated the immunocompetent C57BL/6 model and in the B6.129S7-Rag1^{tm1Mom}/J mice with CL (24). Compared to controls, the administration of CL led to a non-significant increase in tumor growth independent from the presence of T cells and B cells (**Figure 2A**), and to a significantly shorter survival of the mice (for C57BL/6 mice, $p = 0.004$; for B6.129S7-Rag1^{tm1Mom}/J mice, $p = 0.0005$; **Figure 2B**). Administration of CL also reduced the incidence of ascites, both in B6.129S7-Rag1^{tm1Mom}/J and in the C57BL/6 mice (16 and 33% of the B6.129S7-Rag1^{tm1Mom}/J and C57BL/6 mice treated with CL developed ascites, respectively; in comparison to 90% of the untreated B6.129S7-Rag1^{tm1Mom}/J and C57BL/6 mice) (20).

Next, we studied the immunological changes during CL treatment to investigate the underlying mechanisms in detail. Using flow cytometry, we analyzed the immune cells present in peritoneal washings of C57BL/6 mice treated with CL and compared with PBS-treated controls at two predefined time points (T1 and T2, respectively, 23 and 30 days after tumor inoculation). Macrophages were reduced to <1% of CD11b$^+$ cells after the administration of CL, demonstrating they high efficacy of CL in depleting TAMs in the ID8-fLuc ovarian cancer model (**Figure 3A**). In accordance to literature, we observed no significant changes in CD4$^+$ T cells, regulatory T cells (Treg) or conventional dendritic cells (cDC) following CL administration (**Figures 3B–D**) (25). At the first time point, we observed a higher amount of CD8$^+$ T cells and NK cells in CL-treated mice; however, this effect was lost at the second time point (**Figures 3E,F**). The number of CD11b$^+$ cells was significantly reduced upon treatment with CL at the first time point (**Figure 3G**). CL led not only to a significant decrease of TAM, but also a reduction in granulocytic MDSC (gMDSC), plasmacytoid DC (pDC), and B cells (**Figures 3H–J**). Monocytic MDSCs (mMDSC) were the only cell population, which were significantly increased at both time points upon CL treatment (**Figure 3K**). Additionally, we observed a clear significant increase in pro-inflammatory cytokines in ascites, such as interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNFα), and interferon-γ (IFN- γ) (**Figures 4A–D**). This effect was not limited to ascites; we observed similar findings in serum of mice treated with CL (**Figure 4E**). As an additional readout,

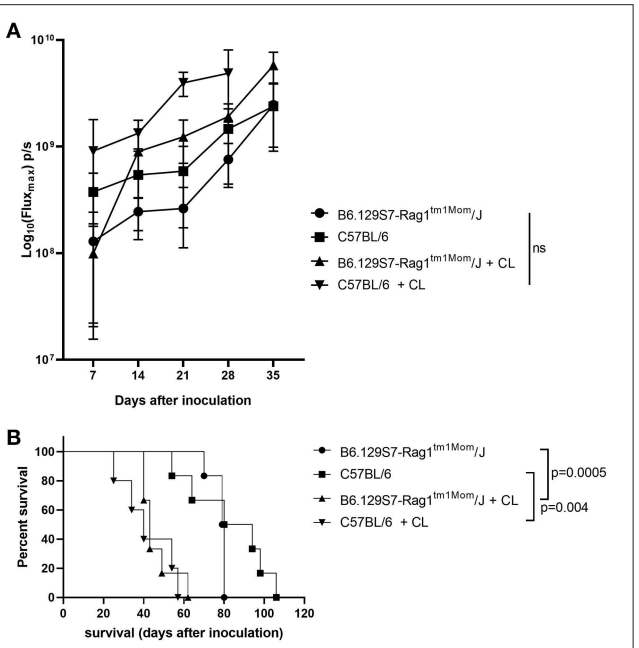

FIGURE 2 | Influence of the innate immune system on tumor growth. **(A)** Evaluation of tumor growth using BLI, Log_{10} transformation of maximal flux in photons per second (p/s) are shown as mean with standard deviation. Tumor growth was increased in both in C57BL/6 mice and B6.129S7-Rag1^{tm1Mom}/J mice when treated with clodronate liposomes (CL) ($n = 6$ mice per group). **(B)** Kaplan-Meier curve showing the survival of B6.129S7-Rag1^{tm1Mom}/J mice and C57BL/6 mice treated with CL. In both groups treatment with CL led to a significant reduction in survival of the mice ($p = 0.0005$ and $p = 0.004$, respectively) ($n = 6$ mice per group).

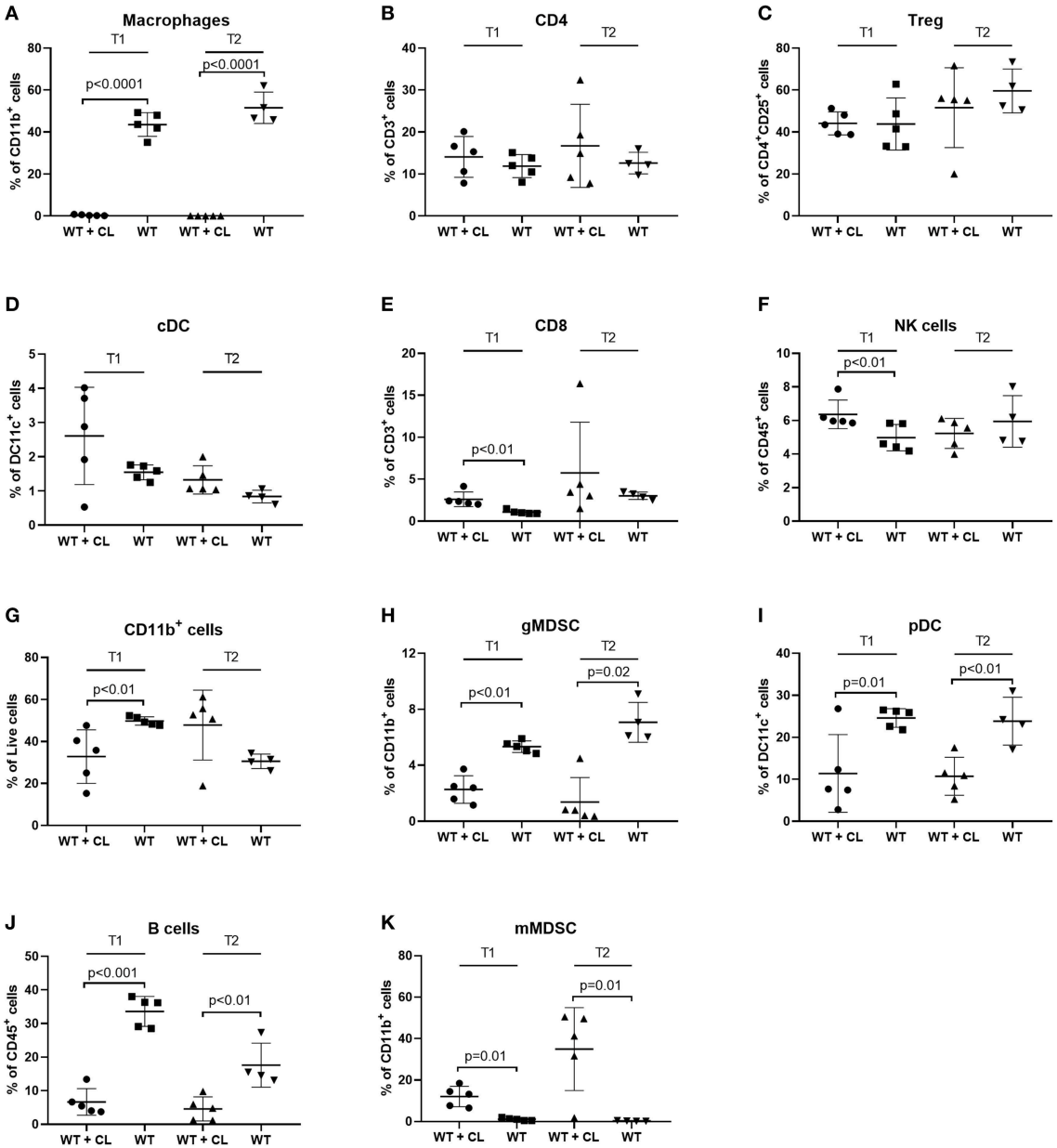

FIGURE 3 | Effect of Clodronate liposomes (CL) on immune cells in the peritoneal cavity of tumor-bearing mice measured by FACS. Changes in immune cells in peritoneal washings during treatment with CL. Immunocompetent animals (C57BL/6) treated with CL (WT + CL) are compared to untreated wild type animals (WT) at two time points (T1 = 23 days after inoculation-T2 = 30 days after inoculation). ($n = 5$ mice per group). **(A)** Treatment with CL led to a relevant depletion of macrophages after treatment with CL to <1% of CD11b$^+$ cells ($p < 0.0001$ for both time points). **(B–D)** No significant changes in CD4$^+$ T cells, regulatory T cells (Treg) or conventional dendritic cells (cDC) were observed. **(E–G)** For CD8$^+$ T cells, natural killer (NK) cells and CD11b$^+$ myeloid cells significant differences [increase of CD8$^+$ T cells and NK cells upon treatment with CL ($p < 0.01$ in both cases) and a reduction in CD11b$^+$ cells in CL treated mice ($p < 0.01$)], were observed on the first time point only. **(H–J)** On both time points we observed a significant decrease in granulocytic myeloid-derived suppressor cells (gMDSC) (T1 $p < 0.01$-T2 $p = 0.02$), plasmacytoid dendritic cells (pDC) (T1 $p = 0.01$-T2 $p < 0.01$) and B cells (T1 $p < 0.001$-T2 $p < 0.01$). **(K)** Monocytic myeloid derived suppressor cells (mMDSC) increased after treatment with CL ($p = 0.01$ for both time points).

we performed IHC staining for Ly6C peritoneal biopsies of mice treated with CL or PBS. At the second time point, we observed an increase of Ly6C$^+$ cells ($p = 0.05$), demonstrating the increased presence of intra-tumoral Ly6C$^+$ MDSC upon CL treatment (**Figures 4F–H**).

Selective Depletion of Innate Immune Cells Using Monoclonal Antibodies

Based on these findings, we performed a selective depletion of TAM, MDSC and NK cells using depleting monoclonal antibodies (mAb). In none of these experiments, we were able

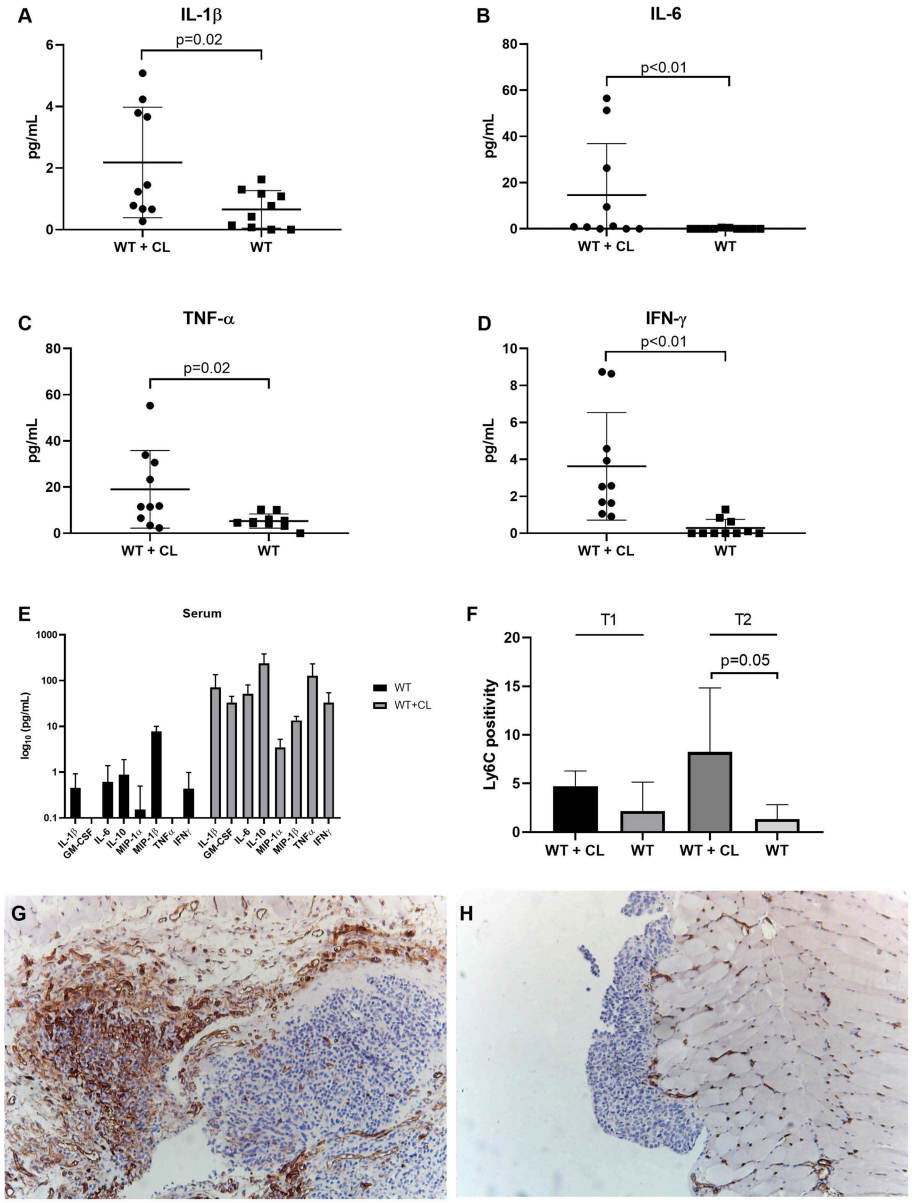

FIGURE 4 | Effect of Clodronate liposomes (CL) on cytokines and tumor-infiltrating MDSC in tumor-bearing mice. Measurement of cytokines in peritoneal washings and serum of C57BL/6 mice treated with CL (WT + CL) are compared to untreated mice. **(A–D)** Changes in cytokines in peritoneal washings due to CL treatment: We observed a significant increase in pro-inflammatory cytokines such as IL-1β (Interleukin), IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFNγ). **(E)** Changes in cytokines in serum due to CL treatment at the second time point (day 30 after inoculation). We observed a significant increase in cytokines such as GM-CSF ($p = 0.0004$), IL-6 ($p = 0.004$), IL-10 ($p = 0.006$), Microphage inflammatory protein 1 (MIP1) α ($p = 0.003$), MIP1β ($p = 0.009$), and IFNγ ($p = 0.008$). **(F)** Using immunohistochemistry, we evaluated the percentage of Ly6C positivity during treatment with CL. Immunocompetent animals (C57BL/6) treated with CL (WT+CL) are compared to untreated wild type animals (WT). At the second time point, 30 days after inoculation we observed a significant higher number of $Ly6C^+$ MDSC cells in the tumor upon treatment with clodronate liposomes ($p = 0.05$). ($n = 5$ mice per group). **(G,H)** Representative Ly6C staining of Immunocompetent animals (C57BL/6) treated with CL (WT+CL) **(G)** and untreated wild type animals (WT) **(H)** at the second time point. Magnification 10x.

to detect significant differences in tumor growth using BLI (**Figure 5A**). Depletion of MDSC using anti-GR1 led to an increase in median survival from 81.5 to 91 days compared to untreated mice. The mice, which received anti-GR1, showed a significant survival advantage compared to the anti-CSF1 treated mice ($p = 0.01$; **Figure 5B**). Selective depletion of TAM using anti-CSF1 (5A1) led, similar to treatment with CL, to a non-significant reduction in median survival from 81.5 days (untreated mice) to 67.5 days (anti-CSF1 treated mice). Of note, treatment with anti-CSF1 depleted ∼70% of TAM (**Figures 5C,D**), which was less profound (61.5% reduction of TAM after treatment with anti-CSF1 compared to the

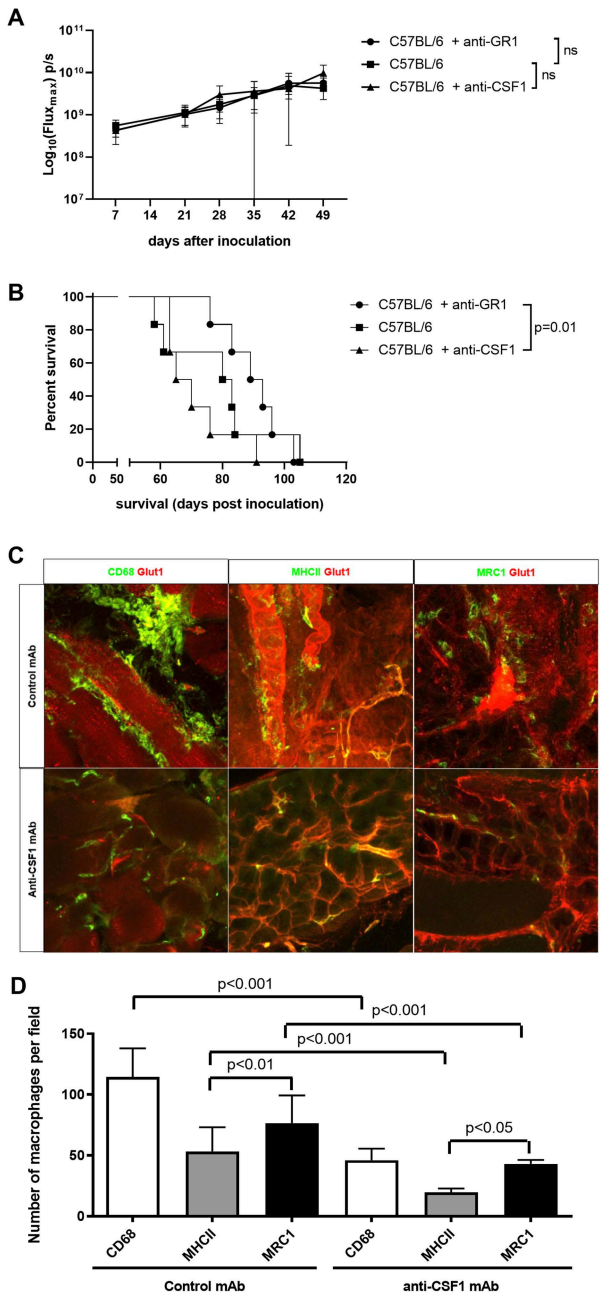

FIGURE 5 | Selective depletion of innate immune cells using monoclonal antibodies (mAb). **(A)** Evaluation of tumor growth using BLI, Log_{10} transformation of maximal flux in photons per second (p/s) are shown as mean with standard deviation. We observed no significant differences in tumor load between the untreated immunocompetent mice (C57BL/6) and the MDSC-depleted mice (C57BL/6 + anti-GR1) or the macrophage depleted mice (C57BL/6 + anti-CSF1) ($n = 6$ mice per group). **(B)** Kaplan-Meier curve of untreated immunocompetent mice (C57BL/6) and the MDSC-depleted mice (C57BL/6 + anti-GR1) or the macrophage depleted mice (C57BL/6 + anti-CSF1). We observed a significantly improved survival in the mice treated with anti-GR1 compared to the mice treated with anti-CSF1 ($p = 0.01$). ($n = 6$ mice per group). **(C)** Immunofluorescent images of tumor biopsies of mice treated with anti-CSF1 or control mAb. In all panes blood vessels were stained for Glut1 in red. In the left pane, CD68 in green was used to stain total

(Continued)

FIGURE 5 | macrophages. In the middle pane, green MHC-II staining was used for M1 macrophages and on the right MRC1 staining in green was used for M2 macrophages. The images at the top represent the mice treated with the control antibody, while the images at the bottom represent the mice treated with anti-CSF-1 (scale bar: 50 μm) ($n = 6$ mice per group). **(D)** Quantitative evaluation of macrophages using immunofluorescent staining. Total macrophages were reduced to less than half due to anti-CSF1 (5A1). Both M1 and M2 macrophages were reduced in the same proportion following pan-macrophages mAb induced depletion ($n = 6$ mice per group).

control antibody) compared to the depletion achieved by CL administration (near complete depletion of TAM). In addition, treatment with anti-CSF1 did not lead to a more favorable macrophage polarization (cytotoxic M1 vs. tumor supportive M2 ratio remained unchanged). Depletion of NK cells using anti-CD122 (TMβ1) did not influence tumor growth or survival of the mice.

Ovarian Cancer (ID8-fLuc) Has a More Indolent Nature in B6.129P2(SJL)-Myd88tm1.1Defr/J Mice

In order to confirm our hypothesis that MDSC-mediated immunosuppression stimulates tumor growth and reduces survival in the ID8-fLuc model, we inoculated B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice and C57BL/6 mice with ID8-fLuc cells. The goal of this experiment was to observe the *in vivo* effect of reduced MDSC-mediated immunosuppression. The Myd88 knock-out mice have a mutation in the Myd88 cytosolic adapter protein, which leads to an impaired immunosuppressive function of MDSC (18, 19). In these B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice we observed a longer median survival after inoculation with ID8-fLuc compared to the wild type mice (C57BL/6) (90 days vs. 76 days, respectively) (**Figure 6A**). The B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice also had a significant delay in the onset of ascites compared to C57BL/6 mice (75 days vs. 63 days, respectively,$-p = 0.01$) (**Figure 6B**). We also observed, in addition to the known reduced function of MDSC in Myd88$^{-/-}$ mice, a significantly reduced presence of mMDSC in peritoneal lavage fluid of B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice (**Figure 6C**). Using IHC we observed a larger tumor volume in wild type mice compared to the B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J counterparts. In addition, we also found a reduced infiltration of Ly6C^{+} MDSC in the tumor of B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice compared to C57BL/6 mice (**Figures 6D,E**). Based on these findings, we can conclude that MDSC support tumor growth and have a negative influence on survival of tumor-bearing mice.

Monocytic MDSC increase as tumor develops in ID8-fLuc model and suppress effector T cell functioning.

Next, we studied the natural evolution of MDSC in the ID8-fLuc ovarian cancer model by assessing the relative numbers of MDSC in ascites over time in tumor-bearing mice and healthy controls. As anticipated from literature, we observed higher numbers of mMDSC in tumor-bearing mice compared to naive mice (26). Additionally, we observed significantly more mMDSC in ascites of mice with end stage disease compared to early stage

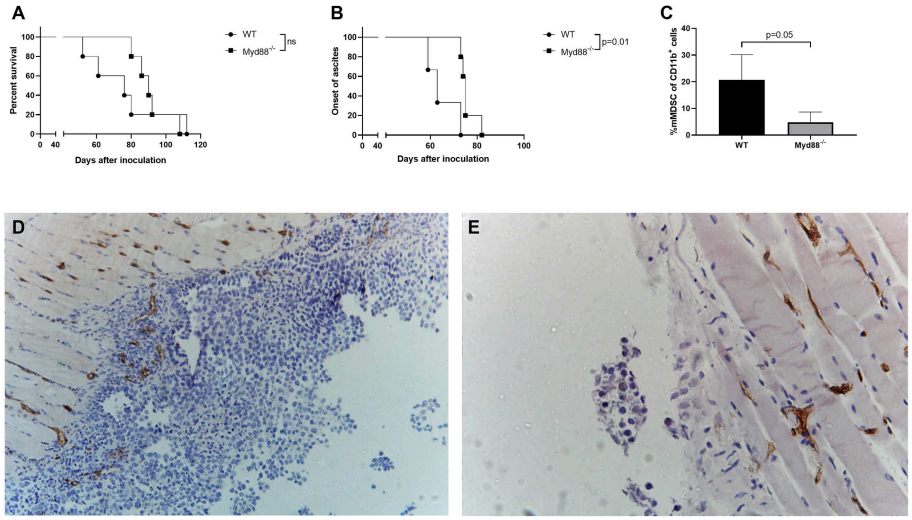

FIGURE 6 | ID8-fLuc induced ovarian cancer has a more indolent nature in Myd88 knockout mice [B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J] compared to wild type (C57BL/6). **(A)** The B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J (Myd88$^{-/-}$) mice had a median survival of 90 days compared to 76 days for C57BL/6 mice (WT). This difference in survival was not statistically significant. ($n = 5$ mice per group). **(B)** Onset of ascites was used here as a surrogate marker for onset of disease symptoms and here we observed a significant longer latency period. ($p = 0.01$) ($n = 5$ mice per group). **(C)** Using FACS we observed significantly less mMDSC in the B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice compared to C57BL/6 mice. **(D,E)** Representative Ly6C staining of C57BL/6 **(D)** and B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J **(E)** mice 8 weeks after inoculation. The C57BL/6 mice displayed macroscopically more peritoneal carcinosis compared to the B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice. In addition, the peritoneal biopsies showed a higher Ly6C positivity in the tumor in the C57BL/6 mice compared to the B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J mice.

disease ($p = 0.02$) (**Figure 7A**). Using immunohistochemistry, we observed an absolute reduction in TAM ($p = 0.004$) and an increase in absolute number of Ly6C$^+$ MDSC ($p = 0.04$) in the tumor over time (**Figures 7B–G**). To study the immunological role of mMDSC in ovarian cancer further, we performed *in vitro* experiments. In these experiments, we evaluated the T cell suppressive capacities of MDSC after stimulation by soluble factors derived from ID8-fLuc cell culture or ascites. Activation of mMDSC by conditioned medium of ID8-fLuc cell culture reduced T cell proliferation ($p = 0.05$), as measured by CFSE (**Figure 8A**). Both mMDSC and gMDSC reduced the number of T cells in co-culture when activated by filtered ascites of tumor bearing mice (**Figure 8B**). Next, we explored the suppressive effect of MDSC on the different T cell subsets using FACS. Co-culture of T cells with mMDSC and gMDSC, led to a reduction in the percentage of CD8$^+$ T cells in the T cell population, even without activation of the MDSC (**Figures 8C,D**). In addition, the number of regulatory T cells (Treg) increased during co-culture with activated gMDSC (**Figure 8E**). Co-culture of T cells with MDSC also led to a strong reduction in de CD8$^+$/CD4$^+$ T cell ratio, irrespective of the activation status of the MDSC (**Figure 8F**). Based on these *in vitro* experiments we can conclude that MDSCs activated by soluble factors present in ascites of ID8-fluc tumor bearing mice induced an unfavorable immune profile with increased regulatory T cells and decreased effector T cells.

DISCUSSION

In this paper we studied the interaction between ovarian cancer and the immune system in the ID8-fLuc ovarian cancer mouse model. In short, we demonstrated that tumor growth and survival of tumor bearing mice is not controlled by the adaptive immune system in the ID8-fLuc model. Tumor growth in B6.129S7-Rag1^{tm1Mom}/J mice, which lack T cells and B cells, was similar to tumor growth in immunocompetent C57BL/6 mice. Survival did not significantly differ between both mice strains. Additionally, depletion of CD8$^+$ T cells did not significantly influence tumor growth in the ID8-fLuc model. There are two main possible explanations for these findings. The first being lack of immunogenicity of the model itself. This is unlikely as multiple studies have shown the antigenicity and immunogenicity of the ID8 model (27–29). Therefore, we hypothesized that the adaptive immune system in the ID8-fLuc model could be rendered anergic. As the behavior of the tumor was very similar in both the specific CD8+ T cell depletion and the B6.129S7-Rag1^{tm1Mom}/J mice, we hypothesized that the innate immune system might play a role in the immunosuppression exerted on the adaptive immune system.

In order to study the role of the innate immune system, macrophages more specifically, we explored the effect of CL in the ID8-fLuc model. Treatment with CL led to a shorter survival both in C57BL/6 mice as in B6.129S7-Rag1^{tm1Mom}/J mice. As CL is considered a dirty drug, which effects are not limited to macrophages only, we investigated the effect of CL treatment on the immune system in the ID8-fLuc model. CL effectively depleted macrophages in the peritoneal cavity of tumor bearing mice. In addition to his, we observed a significant increase in mMDSC and proinflammatory cytokines, which might explain the poor survival of mice treated with CL. We hypothesize that the strong reduction in TAM (to

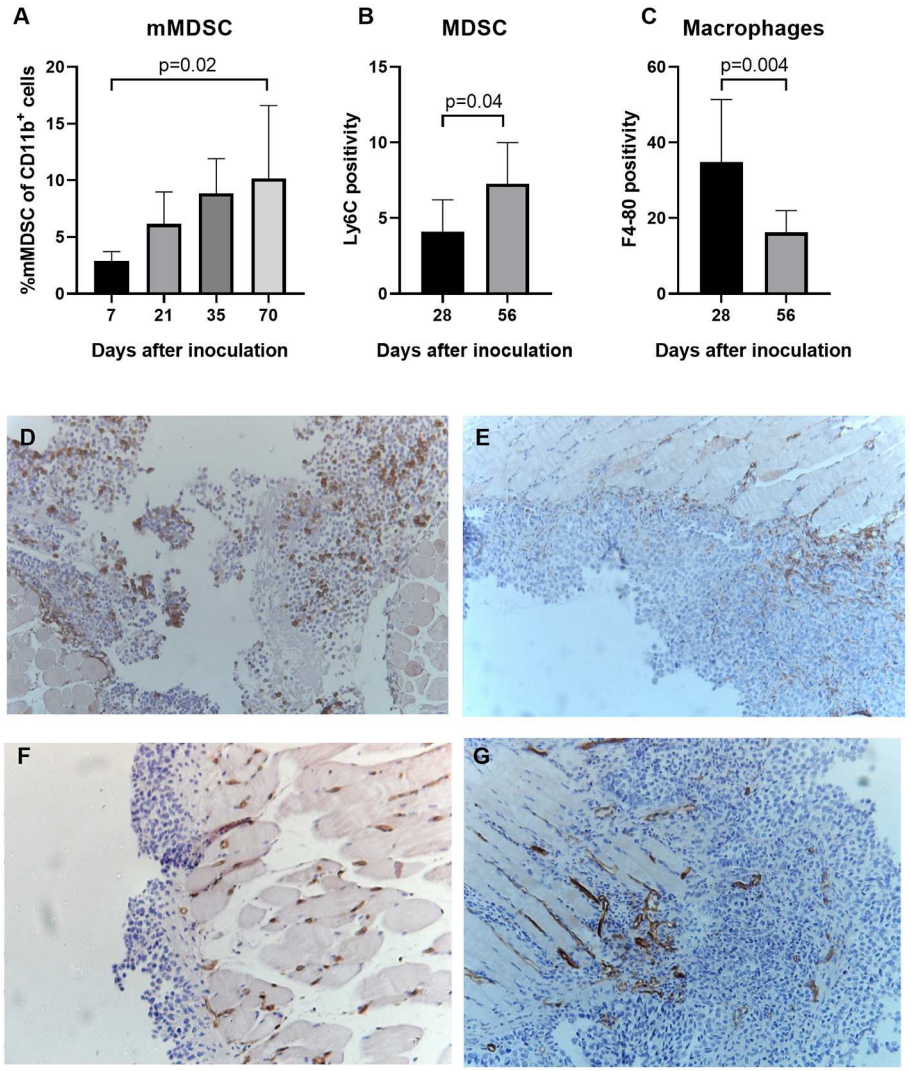

FIGURE 7 | MDSC increase as tumor develops in ID8-fLuc model. **(A)** Using fluorescent activated cell sorting (FACS) we measured the relative number of mMDSC at different time points during tumor development. We observed a significant higher number of monocytic MDSC mice with end stage disease (10 weeks after tumor challenge) compared to mice with early stage disease (1 week after tumor challenge) ($p = 0.02$) ($n = 3$–6 mice per group). **(B,C)** Based on immunohistochemistry for F4-80 and Ly6C we evaluated the presence of, respectively, macrophages and MDSC in the tumor over time. **(B)** We observed a significant decrease in the number of total tumor-associated macrophages over time ($p = 0.004$). **(C)** Parallel to the results using FACS, we observed a significant higher number of Ly6C$^+$MDSC 8 weeks after tumor challenge, compared to 4 weeks after tumor challenge ($p = 0.04$). **(D,E)** Representative images of the F4-80 staining 28 days **(D)** and 56 days **(E)** after inoculation. Magnification x10. **(F,G)** Representative images of the Ly6C staining 28 days **(F)** and 56 days **(G)** after inoculation. Magnification x10.

<1% of CD11b$^+$ cells) disrupts the homeostasis of the tumor microenvironment in the ID8-fLuc model. The observed cytokine reaction could explain the increase in mMDSC, since IL-6 is a known inducer of mMDSC expansion in humans and IL-1b correlates with mMDSC in blood of ovarian cancer patients (30, 31). We also observed a significant increase in Microphage inflammatory protein 1 (MIP1) α and MIP1β, which might have contributed to the recruitment of highly immunosuppressive CCR5$^+$mMDSC (32). The activation of MDSC can lead to an increase in IL-6, IL-10, IL-1β, and IFNγ, creating a feedback loop (32). Based on the assumption that the rise in MDSC caused by the depletion of macrophages by CL was responsible for the detrimental effect on survival of the mice, we performed a more selective depletion experiment. We compared survival and tumor growth of mice treated with anti-CSF1 (selectively TAM depletion), anti-GR-1 (depletion of MDSC), and untreated tumor bearing mice. Selective reduction of GR-1$^+$ MDSCs led to a small survival benefit, as was demonstrated previously by others (33). Survival of mice treated with anti-GR-1 was significantly longer than survival of anti-CSF1 treated mice. Depletion of macrophages by anti-CSF1 was less efficient compared to depletion achieved by CL, which might explain why the effect of anti-CSF1 on survival and tumor growth is less pronounced compared

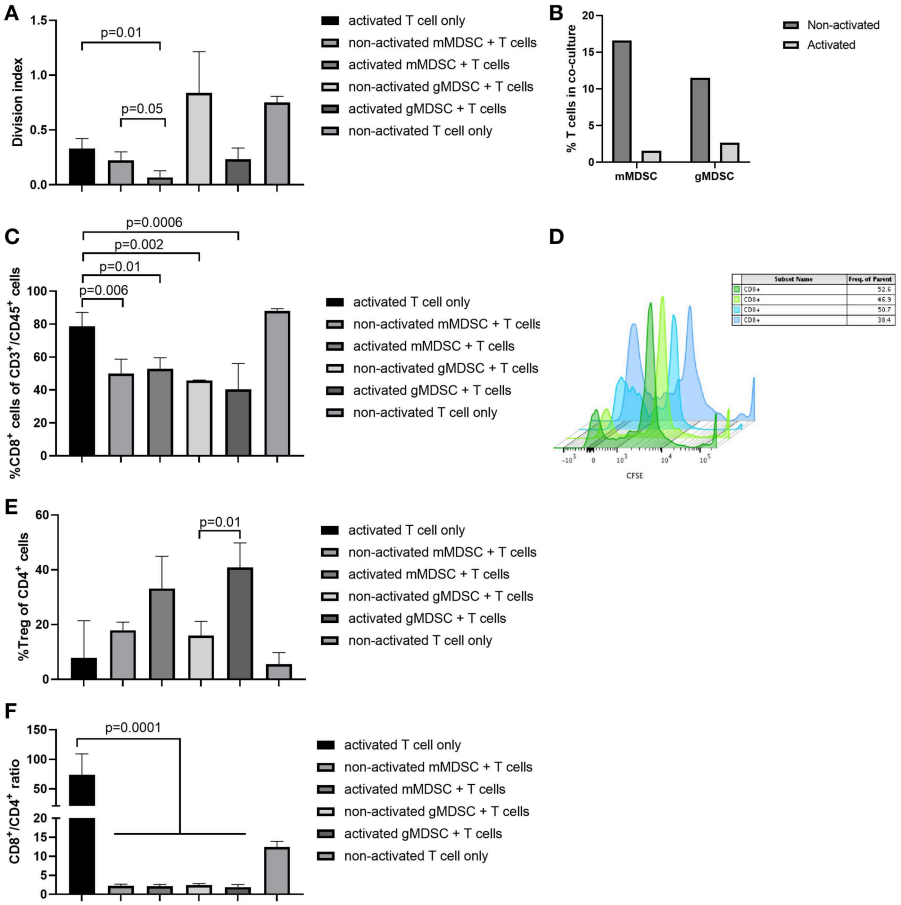

FIGURE 8 | MDSC activated *in vitro* can suppress T cell proliferation and skew the T cell compartment toward a reduction in cytotoxic T cells. **(A)** mMDSC activated by conditioned medium of ID8-fLuc cell culture reduced T cell proliferation significantly ($p = 0.05$) as shown by carboxyfluoresceinsuccinimidyl ester (CFSE). **(B)** Acitvation of mMDSC and gMDSC by filtered ascites led to a strong reduction in the number of live T cells in co-culture. **(C)** FACS analysis of T cells co-cultured *in vitro* with MDSC. Co-culture of mMDSC and gMDSC led to a significant reduction of CD8 positivity. **(D)** MDSC activated by soluble factors in ascites induce a significant reduced T cell proliferation as shown by CSFE dilution. T cells in the presence of non-activated MDSC (blue) are capable of multiple divisions; in contrast T cells co-cultured with activated MDSC display a reduced proliferation (green). **(E)** Activation of gMDSC by filtered ascites led to an increase in regulatory T cells (Treg) ($p = 0.01$). **(F)** Co-culture of both mMDSC and gMDSC skewed the T cell phenotype toward CD4, which led to a reduction in the $CD8^+/CD4^+$ T cells ratio even before activation of MDSC ($p = 0.0001$).

to the CL. To support our hypothesis that MDSC have a negative impact of survival on tumor bearing mice in the ID8-fLuc model, we induced ovarian cancer in B6.129P2(SJL)-$Myd88^{tm1.1Defr}$/J mice. These mice carry a deletion of exon 3 of the myeloid differentiation primary response gene 88 locus, which leads to a reduced (immunosuppressive) function of MDSC. Median survival was longer in the B6.129P2(SJL)-$Myd88^{tm1.1Defr}$/J mice compared to wild type C57BL/6 mice. In addition, onset of disease symptoms (ascites) was significantly delayed in the B6.129P2(SJL)-$Myd88^{tm1.1Defr}$/J mice, supporting our hypothesis.

Next, we investigated the immunosuppressive effects of MDSC on T cells in the context of the ID8-fLuc model *in vitro*. In these experiments, we demonstrated that mMDSC and gMDSC activated by conditioned medium of ID8-fLuc cell culture or filtered ascites, led to a reduction in T cell proliferation and reduced the relative number of effector T cells in co-culture. These findings are supported by Horikawa et al. who demonstrated that MDSC suppress the CD8 T cells in the tumor microenvironment (34), suggesting that MDSC-induced immunosuppression might be one of the drivers of adaptive immunetolerance in ovarian cancer.

It should be noted, however that in an attempt to study the interaction between ovarian cancer and the immune system in a comprehensive way, we decided to use relatively nonspecific tools such as clodronate liposomes and B6.129S7-$Rag1^{tm1Mom}$/J mice. As immunocompetent models for ovarian cancer are scarce, we limited ourselves to the ID8-fLuc model, this is of course also a possible bias. However, we used different methods, which all pointed toward an important role for (monocytic) MDSC in tumor-associated

immunosuppression. In addition, the importance of the innate immune system, MDSC in particular, as a source of immunosuppression is being increasingly recognized in ovarian cancer. Cui et al. were the first to demonstrate a prognostic role for intra-tumoral MDSC in ovarian cancer (16, 34).

In addition, our study underscores the plasticity of the innate immune system and the balanced relationship between the different innate immune cells. A large part of the tumor stroma consists of TAM; therefore, it is not surprising that rash depletion of TAM, leads to a cytokine reaction, which attracts other innate cells to fill this niche. It is also important to note that macrophages and mMDSC originate from the same immature myeloid cells in bone marrow and that mMDSC can differentiate into macrophages (35). Therefore, it is not surprising that such interaction between the innate immune cells exist. Upon treatment with CL mMDSC were attracted to the tumor microenvironment, which led to worse survival of the mice, probably due to a detrimental effect on tumor immune control.

Until recently, tumor immunology research in ovarian cancer has focused mainly on the influence of the adaptive immune system on antitumor immunity (36). Only a limited number of studies have investigated the role of the innate immune system in ovarian cancer. We are the first to demonstrate that the presence of T cells was irrelevant for tumor growth and survival in the ID8-fLuc model. These results suggest that immunosuppression dominates the adaptive immune response in the ID8-fLuc model. In addition, we showed that MDSC are an important source of immunosuppression in ovarian cancer. This is an important finding as clinical immune oncology trials in ovarian cancer are currently focusing on the adaptive immune system. In ovarian cancer, the success has currently been limited to a small number of patients. Targeting MDSC might be a possible strategy to increase the number of patients who respond to immunotherapy. Preclinical studies have detected several possible strategies to deplete or inhibit MDSC, e.g., gemcitabine, 5-FU, ATRA, sunitinib, aspirin etc. (37). However, we believe that further preclinical and translational research is needed to design rational immunotherapeutic approaches in ovarian cancer.

CONTRIBUTION TO THE FIELD STATEMENT

Ovarian cancer is the second most lethal type of gynecological cancer in women with an incidence rate of 12.5 per 100,000 women. Standard therapy consists of extensive surgery in combination with chemotherapy. As tumor-infiltrating lymphocytes have a positive prognostic impact in ovarian cancer, immune checkpoint inhibitors have been put forward as a new treatment modality. However, response was only 10% in monotherapy. According to our findings, this might be explained by underlying immune biology in ovarian cancer. In this paper, we demonstrate that the adaptive immune system is unable to control tumor growth in an ovarian cancer mouse model. We hypothesized that the innate immune system suppresses the adaptive immune response. We show that myeloid-derived suppressor cells (MDSC), increase during the disease course and that MDSC are able to suppress the T cells of the adaptive immune system. In addition, we show that suppression of MDSC function positively influences survival of mice with ovarian cancer. Therefore, we argue that MDSC play an important immunosuppressive role in ovarian cancer and that future studies on immunotherapy should consider combining agents that optimize the T cells response to strategies targeting innate immunosuppression.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

All animals were housed and treated according to the Federation for Laboratory Animal Science Associations guidelines (21). Ethical approval was obtained from the local Ethical Committee at KU Leuven (p075/2014 and p125/2017).

AUTHOR CONTRIBUTIONS

All authors helped write and approved the submitted version of the manuscript. TB designed the study, wrote the manuscript, set-up and performed the *in vivo* experiments, analyzed the data, and performed the statistical analyses. AV helped with the *in vivo* experiments, performed *in vitro* experiments, and scored the IHC. MR provided technical assistance. GT and AVH performed FACS staining and acquisition. TM performed immunofluorescent staining. IV added to the concept of the study. AC supervised the experiments, helped write the manuscript, and conceived the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01273/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. (2016) 66:7–30. doi: 10.3322/caac.21332
2. Karam A, Ledermann JA, Kim JW, Sehouli J, Lu K, Gourley C, et al. Fifth ovarian cancer consensus conference of the gynecologic cancer InterGroup: first-line interventions. *Ann Oncol Off J Eur Soc Med Oncol*. (2017) 28:711–7. doi: 10.1093/annonc/mdx011
3. Ries LAG, Young JL, Keel GE, Eisner ME, Lin YD, Horner M-JD. Cancer survival among adults: US SEER program, 1988-2001. *SEER Surviv Monogr*. (2007) 07-6215:1988–2001.
4. Cancer T, Atlas G. Integrated genomic analyses of ovarian carcinoma. *Nature*. (2011) 474:609–15. doi: 10.1038/nature10166
5. Konecny GE, Wang C, Hamidi H, Winterhoff B, Kalli KR, Dering J, et al. Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. *J Natl Cancer Inst*. (2014) 106:1–8. doi: 10.1093/jnci/dju249
6. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med*. (2003) 348:203–13. doi: 10.1056/NEJMoa020177
7. Bowtell DD, Böhm S, Ahmed AA, Aspuria P-J, Bast RC, Beral V, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer*. (2015) 15:668–79. doi: 10.1038/nrc4019
8. Turner TB, Buchsbaum DJ, Straughn JM, Randall TD, Arend RC. Ovarian cancer and the immune system - the role of targeted therapies. *Gynecol Oncol*. (2016) 142:349–56. doi: 10.1016/j.ygyno.2016.05.007
9. Baert T, Vergote I, Coosemans A. Ovarian cancer and the immune system. *Gynecol Oncol Rep*. (2017) 19:57–8. doi: 10.1016/j.gore.2017.01.002
10. Gaillard SL, Secord AA, Monk B. The role of immune checkpoint inhibition in the treatment of ovarian cancer. *Gynecol Oncol Res Pract*. (2016) 3:1–14. doi: 10.1186/s40661-016-0033-6
11. Leffers N, Daemen T, Helfrich W, Hm B, Bj C, Melief K, et al. Antigen-specific active immunotherapy for ovarian cancer (Review) Antigen-specific active immunotherapy for ovarian cancer. *Cochrane Database Syst Rev*. (2014) 9:CD007287. doi: 10.1002/14651858.CD007287.pub3
12. Baert T, Garg AD, Vindevogel E, Van Hoylandt A, Verbist G, Agonistinis P, et al. *In vitro* generation of murine dendritic cells for cancer immunotherapy: an optimized protocol. *Anticancer Res*. (2016) 36:5793–801. doi: 10.21873/anticanres.11163
13. Reinartz S, Schumann T, Finkernagel F, Wortmann A, Jansen JM, Meissner W, et al. Mixed-polarization phenotype of ascites-associated macrophages in human ovarian carcinoma: correlation of CD163 expression, cytokine levels and early relapse. *Int J Cancer*. (2014) 134:32–42. doi: 10.1002/ijc.28335
14. Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A, et al. A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. *J Ovarian Res*. (2014) 7:19. doi: 10.1186/1757-2215-7-19
15. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. *J Clin Oncol*. (2014) 32:1302–8. doi: 10.1200/JCO.2013.51.4489
16. Cui TX, Kryczek I, Zhao L, Zhao E, Kuick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CTBP2. *Immunity*. (2013) 39:611–21. doi: 10.1016/j.immuni.2013.08.025
17. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell*. (1992) 68:869–77. doi: 10.1016/0092-8674(92)90030-G
18. Hong E-H, Chang S-Y, Lee B-R, Kim Y-S, Lee J-M, Kang C-Y, et al. Blockade of Myd88 signaling induces antitumor effects by skewing the immunosuppressive function of myeloid-derived suppressor cells. *Int J cancer*. (2013) 132:2839–48. doi: 10.1002/ijc.27974
19. Hou B, Reizis B, DeFranco AL. Toll-like receptors activate innate and adaptive immunity by using dendritic cell-intrinsic and -extrinsic mechanisms. *Immunity*. (2008) 29:272–82. doi: 10.1016/j.immuni.2008.05.016
20. Baert T, Verschuere T, Van Hoylandt A, Gijsbers R, Vergote I, Coosemans A. The dark side of ID8-Luc2: pitfalls for luciferase tagged murine models for ovarian cancer. *J Immunother Cancer*. (2015) 3:57. doi: 10.1186/s40425-015-0102-0
21. Guillen J. FELASA guidelines and recommendations. *J Am Assoc Lab Anim Sci*. (2012) 51:311–21.
22. Klerk C, Overmeer R, Niers T, Versteeg H, Richel D, Buckle T, et al. Validity of bioluminescence measurements for noninvasive *in vivo* imaging of tumor load in small animals. *Biotechniques*. (2007) 43:S7–30. doi: 10.2144/000112515
23. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: An open-source platform for biological-image analysis. *Nat Methods*. (2012) 9:676–82. doi: 10.1038/nmeth.2019
24. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods*. (1994) 174:83–93. doi: 10.1016/0022-1759(94)90012-4
25. Ziesberger S, Odermatt B, Marty C, Zehnder-Fjällman A, Ballmer-Hofer K, Schwendener RA. Clodronate-liposome-mediated depletion of tumour-associated macrophages : a new and highly effective antiangiogenic therapy approach. *Br J Cancer*. (2006) 95:272–81. doi: 10.1038/sj.bjc.6603240
26. Fleming V, Hu X, Weber R, Nagibin V, Groth C, Altevogt P, et al. Targeting myeloid-derived suppressor cells to bypass tumor-induced immunosuppression. *Front Immunol*. (2018) 9:398. doi: 10.3389/fimmu.2018.00398
27. Zhang Z, Chen X, Chang X, Ye X, Li Y, Cui H. Vaccination with embryonic stem cells generates effective antitumor immunity against ovarian cancer. *Int J Mol Med*. (2013) 31:147–53. doi: 10.3892/ijmm.2012.1195
28. Liao JB, Ovenell KJ, Curtis EEM, Cecil DL, Koehnlein MR, Rastetter LR, et al. Preservation of tumor-host immune interactions with luciferase-tagged imaging in a murine model of ovarian cancer. *J Immunother Cancer*. (2015) 3:1–9. doi: 10.1186/s40425-015-0060-6
29. Duraiswamy J, Freeman GJ, Coukos G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. *Cancer Res*. (2013) 73:6900–12. doi: 10.1158/0008-5472.CAN-13-1550
30. Wu L, Deng Z, Peng Y, Han L, Liu J, Wang L, et al. Ascites-derived IL-6 and IL-10 synergistically expand CD14+ HLA-DR-/low myeloid-derived suppressor cells in ovarian cancer patients. *Oncotarget*. (2017) 8:76843–56. doi: 10.18632/oncotarget.20164
31. Wang H, Zhao R, Ren H, Zou M, Zhang J, Zhang Y. The expression and association of CD14(+) HLA-DR(Low/-) myeloid-derived suppressor cell-like cells and interleukin-1β in ovarian cancer. *Zhonghua Yi Xue Za Zhi*. (2017) 97:2663–9. doi: 10.3760/cma.j.issn.0376-2491.2017.34.005
32. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. *Front Immunol*. (2018) 9:1310. doi: 10.3389/fimmu.2018.01310
33. Rattan R, Dar S, Rasool N, Giri S, Munkarah AR. Depletion of immunosuppressive myeloid-derived suppressor cells impedes ovarian cancer growth. *Gynecol Oncol*. (2017) 145:213–4. doi: 10.1016/j.ygyno.2017.03.491
34. Horikawa N, Abiko K, Matsumura N, Hamanishi J, Baba T. Expression of vascular endothelial growth factor in ovarian cancer inhibits tumor immunity through the accumulation of myeloid-derived suppressor cells. *Clin Cancer Res*. (2017) 23:587–600. doi: 10.1158/1078-0432.CCR-16-0387
35. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. (2009) 9:162–74. doi: 10.1038/nri2506
36. Ventriglia J, Paciolla I, Pisano C, Cecere SC, Di Napoli M, Tambaro R, et al. Immunotherapy in ovarian, endometrial and cervical cancer: state of the art and future perspectives. *Cancer Treat Rev*. (2017) 59:109–16. doi: 10.1016/j.ctrv.2017.07.008
37. Najjar YG, Finke JH. Clinical perspectives on targeting of myeloid derived suppressor cells in the treatment of cancer. *Front Oncol*. (2013) 3:49. doi: 10.3389/fonc.2013.00049

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Circulating Myeloid Derived Suppressor Cells (MDSC) That Accumulate in Premalignancy Share Phenotypic and Functional Characteristics With MDSC in Cancer

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Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells that accumulate in circulation of cancer patients and at tumor sites where they suppress anti-tumor immunity. We previously reported that in a colon cancer prevention trial of a MUC1 vaccine tested in individuals at increased risk for colon cancer, those who did not mount immune response to the vaccine had higher pre-vaccination levels of circulating MDSC compared to those who did. We also reported that individuals with pancreatic premalignancy, Intraductal Papillary Mucinous Neoplasm (IPMN), had increased circulating levels of MDSC that inversely correlated with spontaneous antibody responses against the pancreatic tumor associated antigen MUC1, abnormally expressed on IPMN. Accumulation of MDSC in cancer and their immunosuppressive role had been well established but their presence in premalignancy was unexpected. In this study we compared MDSC in premalignancy with those in cancer with the hypothesis that there might be differences in the composition of various MDSC subpopulations and their immunosuppressive functions due to different lengths of exposure to disease and/or different tissue microenvironments. In cohorts of patients with premalignant polyps, colon cancer, premalignant IPMN, and pancreatic cancer, we confirmed higher levels of MDSC in premalignancy compared to healthy controls, higher levels of MDSC in cancer compared to premalignancy, but no difference in their subpopulation composition or immunosuppressive capacity. We show that levels of MDSC in premalignancy correlate negatively *in vivo* with spontaneous MUC1-specific antibody responses and *in vitro* with polyclonal T cell proliferation and IFN-γ secretion.

Keywords: colonic adenomas, intraductal papillary mucinous neoplasm (IPMN), tumor antigen mucin 1, immunosurveillance, myeloid derived suppressor cells (MDSC)

INTRODUCTION

Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells that accumulate in cancer, auto-immunity, and some chronic inflammatory conditions (1, 2). They suppress the function of multiple immune effector cells and in particular T cells through multiple mechanisms. MDSC can be divided into two major subtypes based on their cell surface phenotype and morphology: polymorphonuclear MDSC (PMN-MDSC) and monocytic MDSC (M-MDSC). Additional subtypes have been proposed, such as the early-stage MDSC (E-MDSC) that lack both macrophage and granulocyte markers and are present in some disease settings (3). MDSC have been extensively studied as components of the tumor microenvironment. A clear positive association has been reported between peripheral blood MDSC levels and cancer stage in multiple tumor types including malignant myeloma, colon cancer and pancreatic cancer (4–6). PMN-MDSC are the major immunosuppressive population of MDSC found in cancer patients' blood and at the tumor site (7). M-MDSC, although fewer in number, can have higher T cell suppressive capacity on a per cell basis and are involved in promoting tumor metastasis and serving as biomarkers of tumor prognosis (8, 9).

MDSC expansion and maturation is driven by a complicated signal network in which Prostaglandin E2 (PGE2) plays a critical role. The presence of PGE2 in the environment is essential and sufficient to redirect development of dendritic cells (DC) into fully suppressive MDSCs in a concentration dependent manner (10). Multiple signals that control MDSC expansion also induce PGE2 production creating a positive feedback loop between Cyclooxygenase2 (COX2) and PGE2 in MDSC, leading to increased production of immunosuppressive factors such as Indoleamine 2,3-dioxygenase (IDO), IL-10, IL-4R, Arg-1, and PGE2 itself, all closely related to MDSC suppressive functions (11–15). Furthermore, production of PGE2 by MDSC stimulates the expression of C-X-C chemokine receptor type 4 (CXCR4) and Stromal cell-derived factor 1 (CXCL12) responsiveness, facilitating the migration of MDSC into sites of inflammation and tumor (16).

Although MDSC and their role in the tumor microenvironment have been extensively studied there is still little information on MDSC in early cancer or pre-cancer. With the advent of sophisticated diagnostic methods and increased emphasis on early cancer detection, premalignant lesions are routinely identified, providing research material for study and a new opportunity to better understand the role of MDSC throughout cancer development.

Colon cancer develops along the path of progression from non-advanced adenomas to advanced adenomas to colon cancer (17), accumulating oncogenic mutations along the way (18). In clinical practice, most adenomas are diagnosed by colonoscopy and removed, followed by long term surveillance for adenoma recurrence (19). Both adenomas and colon cancer are characterized by overexpression of the hyperglycosylated tumor forms of the tumor associated antigen MUC1 (20). Similarly, over 15% of pancreatic cancers develop from premalignant cysts in the pancreas known as intraductal papillary mucinous neoplasm (IPMN) that are lined by multiple layers of proliferative ductal epithelial cells overexpressing tumor forms of MUC1. In a previously reported prophylactic vaccine clinical trial (21), we administered the MUC1 vaccine to patients with a history of advanced colonic adenomas who are at increased long-term risk for colon cancer (22). The vaccine elicited strong anti-MUC1 IgG responses in 17 of 39 participants. Compared to those vaccine responders and healthy age-matched controls, significantly higher levels of MDSC were found in the PBMC of non-responders prior to vaccination. This was the first observation of an accumulation of MDSC in premalignancy and their apparent negative effect on the immune response. We made the same observation in patients with IPMN (23), showing that in this premalignant disease MDSC can accumulate in the peripheral blood like they do in colon pre-cancer.

We questioned whether MDSC in patients with premalignancy would be the same in the composition of phenotypically defined subpopulations and in their immunosuppressive capacity as MDSCs in cancer patients. We prospectively collected PBMC from two cohorts of patients: Colon Cohort, those diagnosed with premalignant or malignant disease of the colon (colon adenoma vs. colon cancer), and Pancreas Cohort, those diagnosed with premalignant IPMN or pancreatic cancer. In both cohorts, PBMC from patients who were screened and diagnosed as healthy (no adenoma, IPMN, or cancer) served as controls. We examined levels of total MDSC and then separately three MDSC subpopulations, monocytic (M-MDSC), granulocytic (PMN-MDSC) and early (E-MDSC) (4). In both cohorts we saw an increase in the percent of total MDSC and the various subpopulations in premalignancy and in cancer compared to healthy controls, with the levels in cancer being generally higher than in premalignancy. There was no difference in the MDSC subpopulation composition. Like in cancer, MDSC isolated from premalignancy directly suppressed *in vitro* T cell proliferation and IFN-γ production. Indirect evidence of their *in vivo* suppressive activity was reflected in decreased levels of spontaneous anti-MUC1 IgG and increased levels in plasma of PGE2 and its metabolite.

MATERIALS AND METHODS

Patients and Sample Collection

For the Colon Cohort, after informed consent (IRB#0411047), blood samples for patients undergoing colonoscopy or colon surgery were obtained prior to onset of the procedure, along with an epidemiologic questionnaire, and permission to access medical records. Specimens were processed under standard operating procedures of the Pittsburgh Biospecimen Core. The collection was supported by a grant from the Early Detection Research Network (UO1CA152753).

For the Pancreas Cohort, samples were obtained as part of the The Pancreatic Adenocarcinoma Gene Environment Risk (PAGER) Study—a prospective cohort study of patients at risk or having pancreatic disease (IRB# PRO07030072).

PAGER serves as the universal study for enrolling pancreatic cancer cases and diseased controls subjects at the University of Pittsburgh Medical Center (UPMC) by all of the different medical and surgical disciplines involved in the care of benign and malignant pancreatic diseases. It allows for the collection of biospecimens following the standard operating procedures of the Early Detection Research Network (EDRN) along with associated clinical data including a patient questionnaire and access to the subject's clinical records. Blood samples used in this study were collected on patients prior to any treatment including chemotherapy or surgery.

Blood Processing, Plasma, and Live PBMC Preservation

Whole heparinized blood was layered on lymphocyte separation medium (MPbio) and centrifuged at 800 g for 10 min with lowest acceleration and deceleration speed, the same day it was drawn. Plasma was collected of the top of the separation tube and frozen in small aliquots at −20°C. PBMC were collected from the interphase between plasma and separation medium, washed once, resuspended in 80% human serum and 20% DMSO and stored in liquid nitrogen.

MDSC Phenotyping

Previously frozen PBMC were thawed in the 37°C water bath, washed, and stained for Fluorescence Activated Cell Sorter (FACS) analysis with APC labeled anti-human CD11b (BD Biosciences Clone:ICRF44), PE-Texas/Red labeled anti-human CD33 (BD Biosciences Clone:WM53), FITC labeled anti-human HLA-DR (BD Biosciences Clone:G46-6), V450 labeled Anti-human CD14 (BD Biosciences Clone:MϕP9) and PE-Cy7 labeled anti human CD15 (BD Biosciences Clone:HI98). Stained cells were analyzed on IMM Fortessa (BD Bioscience) and data analyzed using FlowJo (v10) software (FlowJo LLC) (21).

MDSC subpopulation phenotypes were defined according to Bronte et al. (4) as follows:

Total MDSC: $CD11b^{+}HLA\text{-}DR^{-/low}$, $CD33^{+}$
PMN-MDSC: $CD11b^{+}HLA\text{-}DR^{-/low}$ $CD33^{+}$ $CD15^{+}$ $CD14^{-}$
M-MDSC: $CD11b^{+}HLA\text{-}DR^{-/low}$ $CD33^{+}$ $CD15^{-}$ $CD14^{+}$
E-MDSC: $CD11b^{+}HLA\text{-}DR^{-/low}$ $CD33^{+}$ $CD14^{-}$ $CD15^{-}$

Anti-MUC1 IgG and IFN-γ Enzyme Linked Immunosorbent Assays (ELISA)

For anti-MUC1 IgG, 96-well microtiter plates (Immulon 4, Thermo-Fisher Scientific, MA) were coated with 1 μg MUC1 100mer peptide (the sequence PDTRPAPGSTAPPAHGVTSAx5 corresponding to five 20aa tandem repeats) in 50 μl Delbecco's PBS (DPBS) per well at 4°C overnight. The plate was then washed 3 times with DPBS and 2.5% bovine serum albumin (BSA) was added in 100 μl DPBS for 1 h at room temperature (RT) as a blocking reagent. The plate was emptied, 50 μl of plasma added at 1:40 dilution and incubated for 1 h at room temperature (RT) on a shaker. The plate was then washed five times with 0.1% tween20 detergent in DPBS. Fifty microliter alkaline phosphatase conjugated with anti-human IgG (Sigma-Aldrich) in 2.5% BSA DPBS was added and the plate incubated for 1 h at RT. The plate was washed again, 100 μl of p-nitrophenyl phosphate (Sigma-Aldrich) added and the plate incubated for 1 h in the dark. The reaction was stopped with 50 μl 0.5 M NaOH. The plates were read at OD 405 nm on the spectrophotometer. Control (no antigen) plate was put through the same reactions except that 50 μl DPBS were added instead of the MUC1 peptide. OD values from the no antigen wells were subtracted from corresponding values on the antigen-coated wells. All samples were tested in triplicates.

IFN-γ ELISA was done according to the manufacturer's protocol (Biolegend, Human IFN-r ELISA sets) with cell supernatants from the cultured T cells added at 1:300 dilution.

Depletion of $CD15^{+}$ Cells From PBMC and T Cell Proliferation Assays

PBMC were isolated from fresh blood and resuspended in 80 μl of MACs buffer [PBS with 0.5% bovine serum albumin (BSA) and 2 mM EDTA]. The cells were then mixed with 20 μl of CD15 MicroBeads (Miltenyi) and incubated at 4°C for 15 min (volume/per 10^{7} total cells). Cells were washed and resuspended in 500 μl of MACs buffer. The cell suspension was applied onto the LS magnetic column (Miltenyi), rinsed by MACs buffer beforehand. The column was washed three times with MACs buffer and unlabeled cells that passed through were collected as $CD15^{+}$ cells-depleted PBMC. T cells were isolated using human Pan T cell isolation beads (Miltenyi) following the manufacturer's protocol with the exception of collecting labeled T cells attached to the column and discarding the unlabeled cells that passed through. Cell purity was analyzed by flow cytometry.

$CD15^{+}$ cells-depleted PBMC or whole PBMC were resuspended at a final concentration of 20×10^{6}/ml in equal amounts of PBS and Cell Trace Yellow (Thermo-Fisher) at a 1:500 dilution, incubated in a 37°C water bath for 8 min and quenched with pre-warmed PBS for another 8 min. The labeled cells were then resuspended in RPMI 1640 medium supplemented with 10% human serum, 0.5% Penicillin-Streptomycin, 0.5% l-glutamine, 0.5% NEAA, 0.5% pyruvate, 200 IU/ml recombinant human IL-2, with TransAct (Miltenyi) in the experiment group, plated in 96-well round bottom plate and placed in the incubator for 4 days at 5% CO_2 and 37°C. T cells in PBS with TransAct were used as a positive control and T cells in medium alone as a negative control. On day 4, cells were harvested and culture supernatants collected for IFN-γ ELISA as described above.

To measure proliferation, cells were suspend in 50 μl FACs buffer with added human Fc receptor blocker (BD Bioscience) at ratio of 1:50, incubated on ice for 20 min and centrifuged at 1,400 rpm for 5 min. Cells were then resuspend with 50 μl mixed antibody solution at 1:50 dilution of anti-CD3-FITC (BD Bioscience) and 1:50 dilution of Ghost (TonBo Bioscience) in FACS buffer and stained for 1 h on ice in the dark. Cells were then washed and resuspended in 0.3 ml FACS buffer and analyzed on IMM Fortessa (BD Bioscience). Gating and analysis were done on software FlowJo v10 (FlowJo LLC). Live T cells were gated as $Ghost^{-}$ $CD3^{+}$ and proliferation was shown by Cell Trace Yellow.

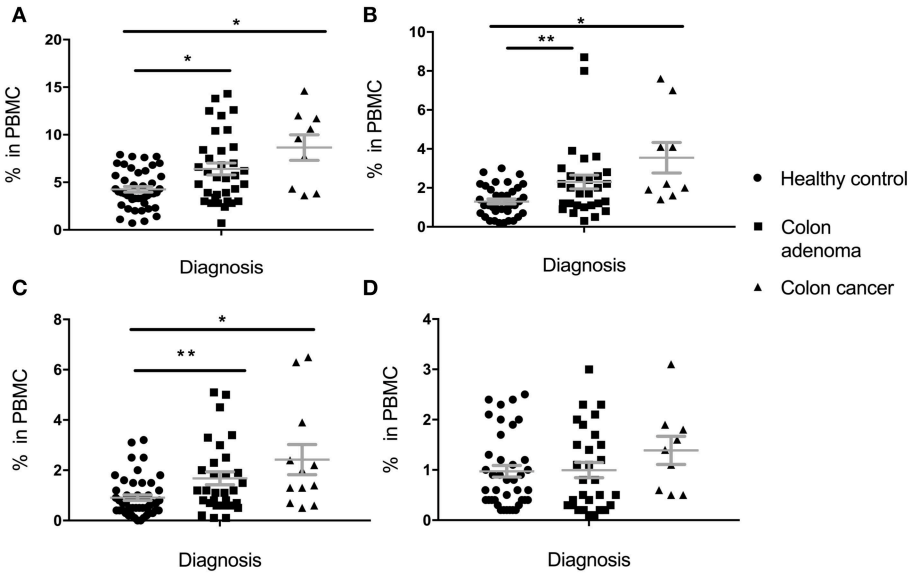

FIGURE 1 | Levels of circulating total MDSC and various MDSC subpopulations in PBMC of individuals diagnosed with adenomas, compared to healthy individuals and individuals with colon cancer. **(A)** Total MDSC; **(B)** PMN-MDSC; **(C)** M-MDSC; **(D)** E-MDSC. Each symbol represents a single individual. Mean with SEM bar for each group is shown in grey. $^{*}p < 0.05$, $^{**}p < 0.01$.

Statistical Analysis

Statistical analyses were performed on GraphPad Prism6 using one-way ANOVA and Student's *t*-test. $P < 0.05$ was considered indicative of statistical significance.

RESULTS

Elevated MDSC Levels in PBMC of Patients With Premalignant Colon Adenomas and Premalignant Pancreatic IPMN

Percentage of total MDSC, PMN-MDSC, M-MDSC, and E-MDSC in PBMC was determined based on cell surface marker expression (see section Materials and Methods). In the Colon Cohort (**Figure 1**), percentages of Total MDSC (**Figure 1A**), PMN-MDSC (**Figure 1B**) and M-MDSC (**Figure 1C**) in premalignant adenoma patients were significantly higher than in healthy controls with all subpopulations still higher in cancer compared to healthy controls. Cells with E-MDSC phenotype (**Figure 1D**) followed a different pattern. They were present at low, normal levels in healthy controls and patients with adenomas. Their percentages appear to increase in colon cancer, albeit not significantly. There was a trend toward slightly higher levels in cancer than in premalignant samples but it did not reach statistical significance (total MDSC: $p = 0.1124$, PMN-MDSC: $p = 0.1171$, M-MDSC: $p = 0.1849$, e-MDSC: $p = 0.2207$).

These results were in great part replicated in the pancreatic cohort (**Figure 2**). Percent of total MDSC was higher in IPMN than in healthy controls and even higher in pancreatic cancer PBMC (**Figure 2A**). This held for all subpopulations, PMN-MDSC (**Figure 2B**), M-MDSC (**Figure 2C**) and E-MDSC (**Figure 2D**). Here again, even though there was a trend toward higher levels in cancer vs. premalignant samples, this was not statistically significant (total MDSC: $p = 0.3303$, PMN-MDSC: $p = 0.6387$, M-MDSC: $p = 0.6262$, E-MDSC: $p = 0.1386$).

In vivo Suppressive Function of MDSC in Premalignancy

Colon adenomas and IPMN express abnormal MUC1 found also in colon and pancreatic cancer. We previously published that many patients with those premalignant conditions, similar to many cancer patients, mount a specific anti-MUC1 antibody response (23, 24). As antibodies are known to play a role in tumor immunosurveillance and MDSC are known to suppress most immune effector mechanisms including B cells, we asked if increases in MDSC we described above could have influenced the ability of individuals with premalignancies to mount anti-MUC1 antibody responses. We tested all individuals in the Colon Cohort (**Figure 3A**) and the Pancreas Cohort (**Figure 3B**) from whom we had both PBMC and plasma saved, for anti-MUC1 IgG. In the Colon Cohort, the adenoma group had the highest average level of anti-MUC1 IgG. As would be expected from a progressively more immunosuppressive microenvironment, as the disease progressed to colon cancer, fewer individuals in those groups made anti-MUC1 IgG. We then paired the percent MDSC with anti-MUC1 IgG level for each patient with adenoma (**Figure 3C**). We found that MDSC levels negatively correlated with the anti-MUC1 IgG levels ($p = 0.0419$, $r = -0.3232$).

We had previously published a similar result in patients with IPMN, which we wanted to confirm in this new Pancreas Cohort and to compare with the Colon Cohort. We had a much smaller number of IPMN patients this time so we combined them with the cancer patients, some of whom were positive for anti-MUC1 IgG. We again see that patients with IPMN and cancer show

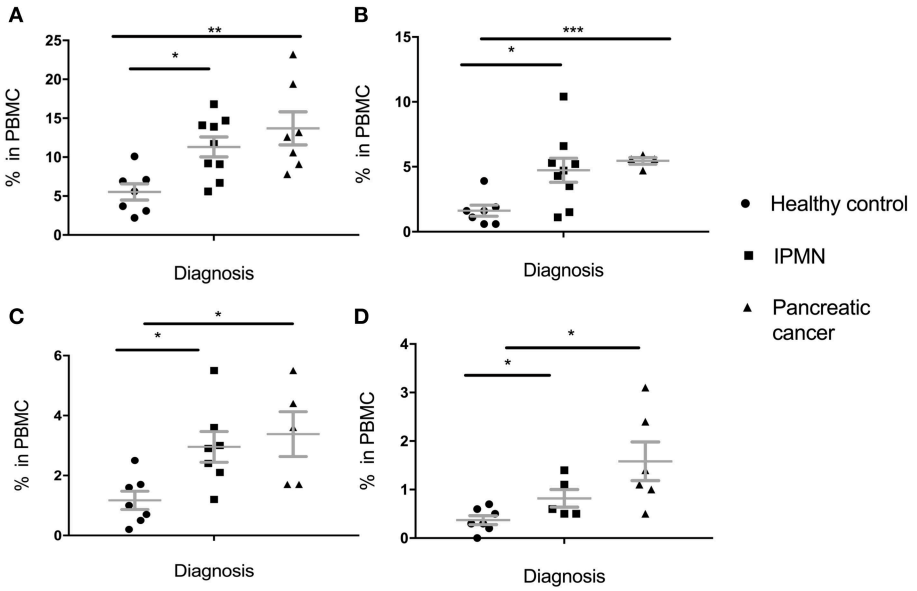

FIGURE 2 | Levels of circulating total MDSC and various MDSC subpopulations in PBMC of individuals diagnosed with premalignant pancreatic intraductal papillary mucinous neoplasms (IPMN) compared to healthy individuals and individuals with pancreatic cancer. **(A)** Total MDSC; **(B)** PMN-MDSC; **(C)** M-MDSC; **(D)** E-MDSC. Each symbol represent a single individual. Mean with SEM bar for each group is shown in grey. $^{*}p < 0.05$, $^{**}p < 0.01$,$^{***}p < 0.001$.

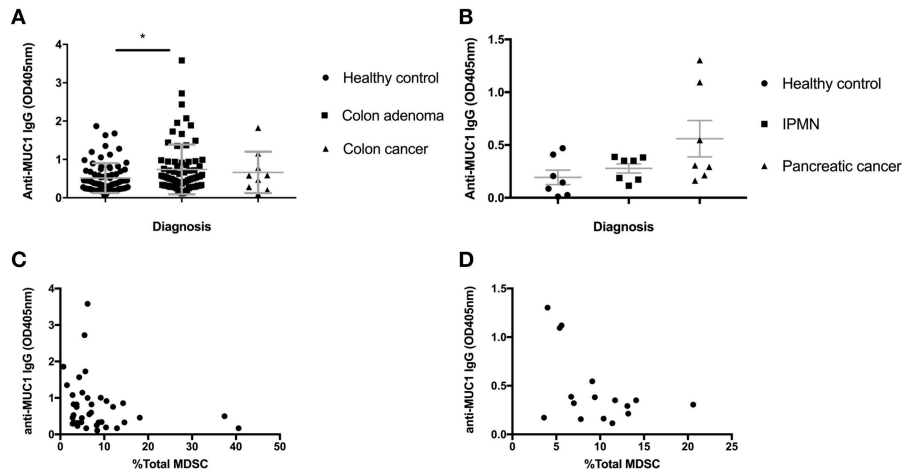

FIGURE 3 | Serum anti-MUC1 IgG levels negatively correlate with MDSC percentages in PBMC in premalignancy. **(A)** IgG levels measured by ELISA in healthy individuals, adenomas and colon cancer. **(B)** IgG levels measured by ELISA in healthy individuals, IPMN and pancreatic cancer. Mean +/– SEM indicated in gray. Analysis was performed using Mann-Whitney test. $^{*}p < 0.05$ **(C)** anti-MUC1 IgG levels in adenomas correlate negatively with the percent of total MDSC in PBMC; **(D)** anti-MUC1 IgG levels in IPMN samples pooled with adenocarcinoma samples show a trend toward negative correlation with the percent of total MDSC in PBMC. Each dot represents a patient; analysis was performed using Spearman correlation.

higher average levels of anti-MUC1 IgG compared to healthy donors and importantly when IgG OD of each patient was paired with the same patient's percent of MDSC (**Figure 3D**), there was a negative correlation ($p = 0.132$, $r = -0.3941$).

In addition to looking at lower IgG levels as indicators of *in vivo* suppressive effects of MDSC, we sought another biomarker of their presence and *in vivo* suppressive function. PGE2 (Prostaglandin E2) largely contributes to the generation of MDSC from immature myeloid cell and their proliferation and acquisition of inhibitory function (25). We measured PGE2 metabolite in sera of all the groups in the Colon and the Pancreas Cohorts (**Figure 4**). We found a significant increase of PGE2M in the IPMN group (**Figure 4B**) ($p = 0.0136$) and a trend toward higher levels in the adenoma group (**Figure 4A**) compared to healthy controls ($p = 0.1139$).

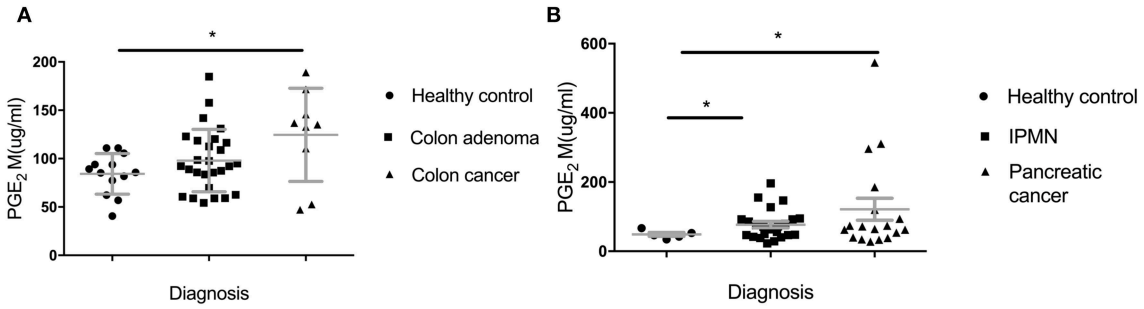

FIGURE 4 | Serum levels of Prostaglandin E2 metabolite (PGE2M). **(A)** Patients with adenomas and colon cancer and healthy controls; **(B)** IPMN and pancreatic cancer compared to healthy controls. PGE_2M concentration was measured by ELISA. Each dot represents an individual patient. Analysis was performed using unpaired student *t*-test. $^*p<0.05$.

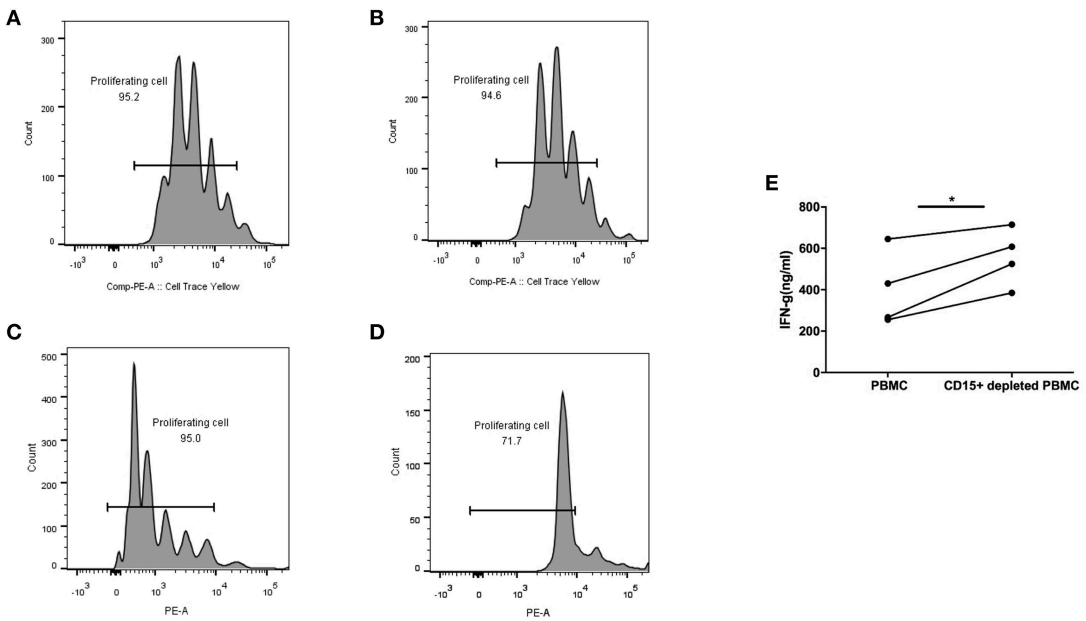

FIGURE 5 | CD15+ MDSC in PBMC from colon adenoma patients suppress their T cell proliferation and Interferon-g production. T cells from Patient 1 **(A,B)** and Patient 2 **(C,D)** in either whole PBMC **(A,C)** or after depletion of CD15+ cells **(B,D)** were activated and their proliferation measured by SFSC dilution 4 days later. **(E)** Interferon-g production by T cells in PBMCs from colon adenoma patients activated in whole PBMC or after depletion of CD15% MDSC, $^*p < 0.05$.

In vitro Suppressive Function of MDSC From Premalignancy

All the above experiments were performed with previously frozen PBMC and plasma. For *in vitro* functional studies of MDSC it was necessary to use fresh blood, which put a limitation on the number of samples we were able to test. We obtained blood from advanced colon adenoma patients one at a time and processed PBMC the same day. We were interested in measuring the function of T cells in each sample in whole blood with MDSC present or after their depletion, which we accomplished by removing $CD15^+$ cells, the majority of which are MDSC. T cells in whole PBMC or MDSC-depleted PBMC were activated with Human IL-2/TransAct (Miltenyi) and cultured for 4 days. T cell proliferation and IFN-γ production were measured and compared between the two groups. **Figure 5** shows two patients with premalignant colonic adenomas. One had 4% MDSC ($CD15^+$) in PBMC (**Figures 5A,B**) and the other had 31% (**Figures 5C,D**). In the case of low to normal numbers of MDSC (4%), T cell proliferation rate was the same in whole PBMC (**Figure 5A**) and after MDSC depletion (**Figure 5B**). On the other hand, in the setting of high MDSC levels (31%), proliferation of T cells was inhibited in whole PBMC (**Figure 5C**) but restored after $CD15^+$ cell depletion (**Figure 5D**).

Furthermore, in another 4/4 PBMC samples from colon adenoma patients, activated T cells in CD15—depleted PBMC secreted higher levels of IFN-γ compared to T cells in whole PMBC ($p < 0.05$).

DISCUSSION

One of the most important findings that came from several decades of basic and preclinical work in tumor immunity and from many failed attempts at immunotherapy, was the highly immunosuppressive nature of the tumor microenvironment, both at the tumor site as well as at a distance, such as in the circulation. We were the first to describe the phenomenon of granulocytes co-sedimenting with white blood cells on a density gradient only in the blood from cancer patients and not from healthy age-matched controls (26). We also showed that numbers of $CD15^+$ cells that we characterized initially as granulocytes in the PBMC correlated inversely with patient survival in three different cancers, colon, breast and pancreas. Importantly, we determined that those were activated granulocytes and we were able to recapitulate *in vitro* their capacity to suppress T cells. Those cells are now known as granulocytic MDSC, or PMN-MDSC ($CD11b^+HLA\text{-}DR^{-/low}$ $CD33^+$ $CD15^+$ $CD14^-$), one of several subpopulations of MDSC responsible for profound suppression of anti-tumor immunity and failure of anti-tumor immunotherapy. The others that we assayed for in this study were subpopulations described in the review by Bronte et al (4), monocytic or M-MDSC ($CD11b^+HLA\text{-}DR^{-/low}$ $CD33^+$ $CD15^-$ $CD14^{+)}$ and early, or E-MDSC ($CD11b^+HLA\text{-}DR^{-/low}$ $CD33^+$ $CD14^-$ $CD15^{-)}$. We also looked at the entire heterogeneous population that we referred to as Total MDSC ($CD11b^+HLA\text{-}DR^{-/low}$, $CD33^{+)}$.

MDSC have been reported in several chronic inflammatory diseases (27, 28) but it was only very recently that they were also seen to play immunoinhibitory role in premalignant disease. We first observed their presence in the PBMC of patients with premalignant pancreatic disease, IPMN (29), and later also in patients with premalignant colonic polyps. In the latter, their presence in the PBMC correlated with the inability to respond to a vaccine based on the MUC1 antigen abnormally expressed on colonic polyps and colon cancer, which was being tested for colon cancer prevention (21).

Our observation that MDSC are present in the premalignant as well as the malignant tumor microenvironment begged the question of whether they shared some or all of their phenotypic and functional characteristics. We expected that exposure of MDSC to the premalignant microenvironment would have been of a shorter duration than exposure to the entire process of tumor development and that this would make MDSC in premalignancy in some way different than those described in tumors. While we did not exhaust all the possible comparisons, we can conclude from data obtained in this study that in both premalignant and malignant disease, all phenotypically defined MDSC populations are present and they are immunosuppressive. The only difference appears to be quantitative with the higher numbers generally present in cancer patients. We can also conclude from our *in vitro* T cell experiments that PMN-MDSC are the main immunosuppressive population in these two cancers as depletion of $CD15^+$ cells that spares M-MDSC, eliminates most of the suppression of T cell proliferation and interferon production.

As much as we did not see significant differences between MDSC in premalignancy vs. cancer, we conclude that both conditions can lead to their accumulation and their equally immunosuppressive phenotype. Depletion of these cells, which is a goal of several pharmaceutical companies working on potential reagents that could be used for such a purpose, might be considered not only for improving cancer outcome but also for reducing the risk of progression from premalignant disease to cancer. Furthermore, our hypothesis that we might find differences due to among other factors, the length of time that the premalignant lesion has been in the body compared to cancer, might be more applicable to T cells than MDSCs. We showed in our earlier publication (21), and again in this paper, that when removed from the influence of MDSC, T cells in premalignancy regain their normal proliferation and IFN-γ production. This is not the case with T cells from cancer patients that in most cases remain exhausted and dysfunctional (30, 31). Thus, rescuing T cells in premalignancy by removing MDSC or countering their effects in other ways, may give much better results than similar manipulations in cancer.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Pittsburgh Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocols #0411047 and #PRO07030072 were approved by the University of Pittsburgh IRB.

AUTHOR CONTRIBUTIONS

PM performed all the experiments with the help from PB and JM, analyzed results and wrote the first draft of the manuscript. The study was conceived by OF, RS, and RB and supervised by OF. The manuscript was reviewed, revised, and edited by all authors.

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REFERENCES

1. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W, et al. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. *Proc Natl Acad Sci USA*. (2011) 108:17111–16. doi: 10.1073/pnas.1108121108
2. Sica A, Massarotti M. Myeloid suppressor cells in cancer and autoimmunity. *J Autoimmun*. (2017) 85:117–25. doi: 10.1016/j.jaut.2017.07.010
3. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol*. (2016) 37:208–20. doi: 10.1016/j.it.2016.01.004
4. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun*. (2016) 7:12150. doi: 10.1038/ncomms12150
5. Umansky V, Sevko A, Gebhardt C, Utikal J. Myeloid-derived suppressor cells in malignant melanoma. *J Dtsch Dermatol Ges*. (2014) 12:1021–7. doi: 10.1111/ddg.12411
6. Li W, Wu K, Zhao E, Shi L, Li R, Zhang P, et al. HMGB1 recruits myeloid derived suppressor cells to promote peritoneal dissemination of colon cancer after resection. *Biochem Biophys Res Commun*. (2013) 436:156–61. doi: 10.1016/j.bbrc.2013.04.109
7. Youn J-I, Gabrilovich DI. The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. *Eur J Immunol*. (2010) 40:2969–75. doi: 10.1002/eji.201040895
8. Pergamo M, Miller G. Myeloid-derived suppressor cells and their role in pancreatic cancer. *Cancer Gene Ther*. (2017) 24:100–5. doi: 10.1038/cgt.2016.65
9. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol*. (2018) 19:108–19. doi: 10.1038/s41590-017-0022-x
10. Obermajer N, Kalinski P. Generation of myeloid-derived suppressor cells using prostaglandin E2. *Transplant Res*. (2012) 1:15. doi: 10.1186/2047-1440-1-15
11. Eliopoulos AG, Dumitru CD, Wang C-C, Cho J, Tsichlis PN. Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals. *EMBO J*. (2002) 21:4831–40. doi: 10.1093/emboj/cdf478
12. Sierra JC, Hobbs S, Chaturvedi R, Yan F, Wilson KT, Peek RM, et al. Induction of COX-2 expression by Helicobacter pylori is mediated by activation of epidermal growth factor receptor in gastric epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. (2013) 305:G196–203. doi: 10.1152/ajpgi.00495.2012
13. Hou Z, Falcone DJ, Subbaramaiah K, Dannenberg AJ. Macrophages induce COX-2 expression in breast cancer cells: role of IL-1β autoamplification. *Carcinogenesis*. (2011) 32:695–702. doi: 10.1093/carcin/bgr027
14. Kohanbash G, McKaveney K, Sakaki M, Ueda R, Mintz AH, Amankulor N, et al. GM-CSF promotes the immunosuppressive activity of glioma-infiltrating myeloid cells through interleukin-4 receptor-α. *Cancer Res*. (2013) 73:6413–23. doi: 10.1158/0008-5472.CAN-12-4124
15. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J Exp Med*. (2005) 202:931–9. doi: 10.1084/jem.20050715
16. Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P. PGE(2)-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. *Cancer Res*. (2011) 71:7463–70. doi: 10.1158/0008-5472.CAN-11-2449
17. Schoen RE, Corle D, Cranston L, Weissfeld JL, Lance P, Burt R, et al. Is colonoscopy needed for the nonadvanced adenoma found on sigmoidoscopy? *Gastroenterology*. (1998) 115:533–41. doi: 10.1016/S0016-5085(98)70132-5
18. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. (1990) 61:759–67. doi: 10.1016/0092-8674(90)90186-I
19. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR, et al. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*. (2012) 143:844–57. doi: 10.1053/j.gastro.2012.06.001
20. Mukherjee P, Pathangey LB, Bradley JB, Tinder TL, Basu GD, Akporiaye ET, et al. MUC1-specific immune therapy generates a strong anti-tumor response in a MUC1-tolerant colon cancer model. *Vaccine*. (2007) 25:1607–18. doi: 10.1016/j.vaccine.2006.11.007
21. Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. *Cancer Prev Res*. (2013) 6:18–26. doi: 10.1158/1940-6207.CAPR-12-0275
22. Click B, Pinsky PF, Hickey T, Doroudi M, Schoen RE. Association of colonoscopy adenoma findings with long-term colorectal cancer incidence. *JAMA*. (2018) 319:2021–31. doi: 10.1001/jama.2018.5809
23. Beatty PL, van der Geest R, Hashash JG, Kimura T, Gutkin D, Brand RE, et al. Immunobiology and immunosurveillance in patients with intraductal papillary mucinous neoplasms (IPMNs), premalignant precursors of pancreatic adenocarcinomas. *Cancer Immunol Immunother*. (2016) 65:771–8. doi: 10.1007/s00262-016-1838-1
24. Finn OJ, Gantt KR, Lepisto AJ, Pejawar-Gaddy S, Xue J, Beatty PL. Importance of MUC1 and spontaneous mouse tumor models for understanding the immunobiology of human adenocarcinomas. *Immunol Res*. (2011) 50:261–8. doi: 10.1007/s12026-011-8214-1
25. Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood*. (2011) 118:5498–505. doi: 10.1182/blood-2011-07-365825
26. Schmielau J, and Finn O. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Cancer Res*. (2001) 61:4756–60.
27. Dorhoi A, Du Plessis N. Monocytic myeloid-derived suppressor cells in chronic infections. *Front Immunol*. (2017) 8:1895. doi: 10.3389/fimmu.2017.01895
28. Wang T, Wen Y, Fan X. Myeloid-derived suppressor cells suppress CD4+ T cell activity and prevent the development of type 2 diabetes. *Acta Biochim Biophys Sin*. (2018) 50:362–9. doi: 10.1093/abbs/gmy014
29. Beatty PL, Narayanan S, Gariépy J, Ranganathan S, Finn OJ. Vaccine against MUC1 antigen expressed in inflammatory bowel disease and cancer lessens colonic inflammation and prevents progression to colitis-associated colon cancer. *Cancer Prev Res*. (2010) 3:438–46. doi: 10.1158/1940-6207.CAPR-09-0194
30. Hamid O, Robert C, Daud A, Hodi FS, Hwu W-J, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. (2013) 369:134–44. doi: 10.1056/NEJMoa1305133
31. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol*. (2013) 25:214–21. doi: 10.1016/j.coi.2012.12.003

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REVIEW
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Lipid Metabolic Pathways Confer the Immunosuppressive Function of Myeloid-Derived Suppressor Cells in Tumor

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Myeloid-derived suppressor cells (MDSCs) play crucial roles in tumorigenesis and their inhibition is critical for successful cancer immunotherapy. MDSCs undergo metabolic reprogramming from glycolysis to fatty acid oxidation (FAO) and oxidative phosphorylation led by lipid accumulation in tumor. Increased exogenous fatty acid uptake by tumor MDSCs enhance their immunosuppressive activity on T-cells thus promoting tumor progression. Tumor-infiltrating MDSCs in mice may prefer FAO over glycolysis as a primary source of energy while treatment with FAO inhibitors improved anti-tumor immunity. This review highlights the immunosuppressive functions of lipid metabolism and its signaling pathways on MDSCs in the tumor microenvironment. The manipulation of these pathways in MDSCs is relevant to understand the tumor microenvironment therefore, could provide novel therapeutic approaches to enhance cancer immunotherapy.

Keywords: MDSCs, lipid metabolism, FAO-OXPHOS, immunosuppressive, cancer immunotherapy

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INTRODUCTION

Myeloid-derived suppressor cells (MDSCs) are pathologically activated cells displaying an exceptional immunosuppressive ability (1, 2). They rapidly expand in cancer, trauma, infectious, autoimmune, and graft vs. host disease (3–8). MDSCs are phenotypically similar to monocytes and neutrophils, thus are further divided into two subsets; monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs) respectively (9, 10). Generally, the cell surface markers for MDSCs include Gr1 and CD11b in mice (11, 12). The M-MDSCs are characterized by $CD11b^{+}Ly6C^{high}Ly6G^{-}$ unlike PMN-MDSCs which are $CD11b^{+}Ly6C^{low}Ly6G^{+}$ (10, 11). Human M-MDSCs on the other hand, are $CD33^{high}CD14^{+}CD15^{+}/CD66b^{-}HLA\text{-}DR^{-/low}$, whereas PMN-MDSCs are characterized by $CD33^{dim}CD14^{-}CD15^{+}/CD66b^{+}HLA\text{-}DR^{-}$ (13, 14). More importantly, these cells potently suppress innate and adaptive immunity. Thus, are considered a promising therapeutic target in cancer immunotherapy.

Metabolic reprogramming has been reported to be a crucial factor in the alteration of MDSCs function (15–20). Lipids which maintain cell membrane integrity, homeostasis, signaling, and healthy performance have been implicated to modulate the function of MDSCs (21–23). Recently, uncontrolled lipid accumulation was found to be higher in MDSCs from cancer patients and

mice with an established tumor compared with tumor-free counterparts (24–26). This increased the immunosuppressive activity in the hyperlipidemic tumor-bearing mice and impaired T-cell activation (24, 25, 27). In this review, we discuss the roles of lipid in modulating MDSCs function and its related metabolic pathways. Further understanding of the biochemical pathways involved in lipid manipulation of MDSCs is pertinent to understand the tumor microenvironment and improve chemo- and immuno-therapies.

AN OVERVIEW OF LIPID METABOLISM IN MDSCS

Acetyl CoA, a major intermediate in several biochemical processes plays a pivotal role in lipid metabolism. It is the primary building blocks for biosynthesis of fatty acid, cholesterol, and the end product of fatty acid oxidation (FAO). Lipid catabolism involves the oxidation of long-chain fatty acids, which takes place in the mitochondrial via the transportation of lipids from the cytosol by the carnitine palmitoyltransferase system. Carnitine palmitoyltransferase 1 (CPT1) catalyzes the rate-limiting step in FAO. During fatty acid oxidation, the continuous elimination of 2-carbon units from the β-position of fatty acyl-CoA molecule produces acetyl CoA which sustains oxidative phosphorylation (OXPHOS) and tricarboxylic acid cycle (TCA) in the cell (28, 29). In contrast to FAO, synthesis of fatty acid occurs in the cytosol; commencing with the carboxylation of acetyl CoA to malonyl CoA in an ATP-dependent manner catalyzed by acetyl CoA carboxylase 1 (ACC1), the reaction rate determining enzyme (**Figure 1**). This is followed by the condensation of another molecule of acetyl CoA with the malonyl CoA to produce saturated long chain fatty acids in a process catalyzed by fatty acid synthase (FASN). These steps lead to the formation of other complex lipids like phospholipids, cholesterol esters, and triglycerides.

ENERGY METABOLIC PATHWAYS OF MDSCS

It is important to note that nearly all major biomolecules (carbohydrates, proteins, and lipids) are converted to a common intermediate in the form of acetyl CoA. Acetyl CoA can be further oxidized to CO_2 or take part in some other biosynthetic pathways as required by the cells. Production of adenosine triphosphate (ATP) to enhance cellular functions, survival, and synthesis of intermediates allowing for cellular growth and proliferation by immune cells rely on various energy metabolic pathways (30, 31). Interconnection of metabolic network (glycolysis, the TCA cycle, and OXPHOS) plays a crucial role in fulfilling these energy needs (**Figure 1**). Glycolysis occurs in the cytosol while the TCA cycle and OXPHOS are restricted to the mitochondria. Glycolysis commences with glucose uptake from the extracellular environment via GLUT; glucose is then phosphorylated to glucose-6- phosphate (G6P) by hexokinases. G6P is further processed to pyruvate via multiple enzyme-catalyzed reactions during which it reduces NAD^+ to NADH to yield 2 molecules of ATP. Under normoxia, glycolysis-derived pyruvate is converted into acetyl-CoA in a reaction regulated by pyruvate dehydrogenase (PDH) complex. This acetyl CoA condenses with oxaloacetate to form citrate in a reaction catalyzed by citrate synthase in the TCA cycle. This cycle produces NADH and FADH2 and transfers electrons generated through the electron transport chain (ETC) to fuel OXPHOS to yield 30–36 molecules of ATP per molecule of glucose. Cells can also use fatty acid through FAO, which yields acetyl-CoA to sustain the TCA cycle and OXPHOS; this can facilitate the generation of substantial amounts of ATP (over a 100 ATP molecules per molecule of palmitate). Most importantly, cells to a varying extent can select their preferred metabolic pathways among several available intermediates to produce ATP. In the immune cells, nutrient, and oxygen availability which can be controlled by growth factors and cytokines, as well as important receptor signaling events, regulate the metabolic fate of these cells.

Certain key enzymes derived from MDSCs are important to their suppressive role, these enzymes deplete the essential amino acids required for T-cell function and proliferation (32). Increased arginase 1 (ARG1) expression in MDSCs depletes L-arginine needed for T-cell functions (33). Also, MDSCs accumulation deplete L-cysteine levels via its sequestration and consumption (34). The depletion of these amino acids results in downregulation of ζ-chain in the T cell receptor (TCR) thus inhibiting proliferation of antigen-specific T-cells. Similarly, MDSCs express the inducible enzyme Indoleamine 2, 3-dioxygenase (IDO), which catalyzes tryptophan metabolism through the kynurenine pathway (35, 36). Thus, IDO expression leads to tryptophan deprivation and induces regulatory T-cells (Tregs) expansion which represses T-cells (37, 38). While the pivotal role of nitrogen metabolism in mediating the immunosuppressive function of MDSCs on T-cells in tumors is well-established (32), little is known about other metabolic pathways in these cells. Carbon metabolism (glycolysis, pentose phosphate pathway (PPP), TCA, FAO pathways) and its crosstalk with nitrogen metabolism during MDSCs maturation in tumors need to be expounded.

FAO and glycolysis are crucial pathways in tumor growth (39), however, it is not known whether MDSCs prefer FAO over glycolysis. It was previously reported that tumor-infiltrating MDSCs (M-MDSCs and PMN-MDSCs) in comparison with peripheral MDSCs and murine myeloid cells prefer FAO as their energy sources (27). This was deduced from the observed elevated mitochondrial mass, increased oxygen consumption rate (OCR) and upregulation of crucial FAO regulatory enzymes [acyl-CoA dehydrogenase (ACADM), CPT1, 3-hydroxyacyl-CoA dehydrogenase (HADHA), and peroxisome proliferator-activated receptor gamma coactivator 1-beta (PGC1β)] in PMN-MDSCs. The study revealed a correlation between expression of FAO genes (such as CPT1 and HADHA) and fatty acid uptake in patient-derived tumor-infiltrating MDSCs (22, 27). In the study, both extracellular acidification rate (ECAR) and OCR were elevated, indicating an overall metabolic alteration. Although the ratio of OCR/ECAR was increased, suggesting that FAO may be

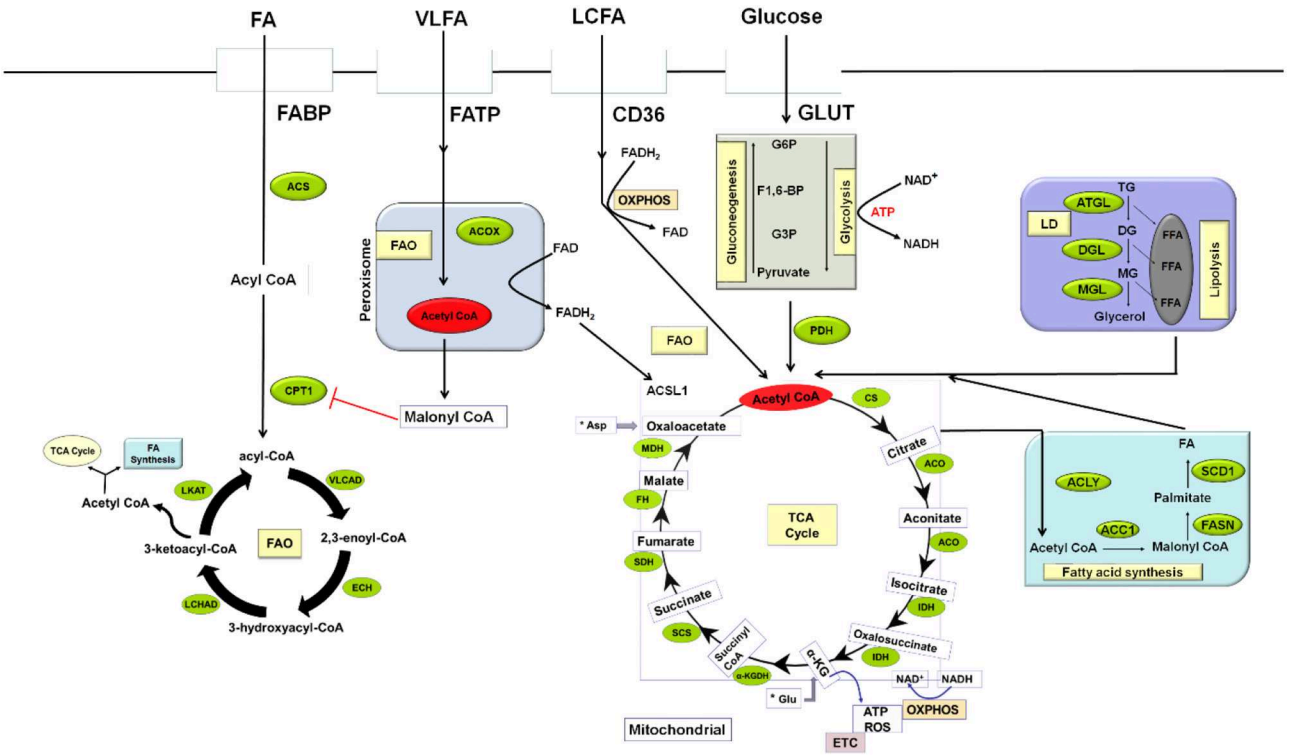

FIGURE 1 | An overview of MDSCs Lipid Metabolism in a tumor environment. Lipid metabolism in MDSCs can undergo two processes: fatty-acid synthesis and fatty-acid β-oxidation. Fatty acid synthesis takes place in the cytosol while β-oxidation occurs in the mitochondrial. Several metabolic networks regulate the activation and survival of MDSCs to enhance tumor proliferation. Glycolysis, the breakdown of glucose to pyruvate with the concomitant release of ATP taking place in the cytosol is the major source of energy to most cells. In the mitochondrial, PDH converts pyruvate to acetyl CoA, the central dogma of metabolism which has several metabolic fates, including TCA cycle, oxidative phosphorylation, and fatty acid biosynthesis. ACC1, Acetyl CoA carboxylase; ACLY, ATP citrate lyase; ACO, Aconitase; ACOX, Acyl coA oxidase; ACS, Acyl CoA synthase; ACSL1, long-chain acyl-CoA synthetase isoform 1; Asp, Aspartate; ATGL, Adipose triglyceride lipase; ATP, Adenosine triphosphate; CD36, Cluster of differentiation 36; CPT, Carnitine palmitoyltransferase 1; DG, Diglyceride; DGL, Diglyceride lipase; ECH, 2, 3-enoyl-CoA hydratase; ETC –Electron transport chain; F1,6-BP, Fructose-1,6-bisphosphate; FA, Fatty acid; FABP, Fatty acid-binding protein; FAD, Flavin adenine dinucleotide; FADH2, Reduced FAD; OXPHOS, Oxidative phosphorylation; FAO, Fatty acid oxidation; FASN, Fatty acid synthase; FATP, Fatty acid transport protein; FFA, Free fatty acid; FH, Fumarate hydratase; G3P, Glyceraldehyde-3-phosphate; Glu, Glutamate; GLUT, Glucose transporter; GP6, Glucose-6-phosphate; IDH, Isocitrate dehydrogenase; LCHAD, Long-chain 3-hydroxyacyl-CoA dehydrogenase; LD, Lipid droplet; LKAT, long chain 3-ketoacyl-CoA thiolase; MDH, Malate dehydrogenase; MG, Monoglyceride; MGL, Monoglyceride lipase; NAD, Nicotinamide adenine dinucleotide; PDH, Pyruvate dehydrogenase; ROS, Reactive Oxygen species; SCD1, Stearoyl-CoA desaturase 1; SCS, Succinyl CoA synthase; SDH, Succinate dehydrogenase; TCA, Tricarboxylic acid; TG, Triglyceride; VLCAD, Very-long-chain acyl-CoA dehydrogenase; VLFA, Very long chain fatty acid; LCFA, Long chain fatty acid; α-KG, Alpha-ketoglutarate; α-KGDH, Alpha ketoglutarate dehydrogenase.

preferred to glycolysis in tumor-infiltrating MDSCs from Lewis lung carcinoma.

In addition, Jian et al., recently reported that ECAR and glycolytic enzymes are upregulated in total MDSCs. Whereas, PMN-MDSCs were observed to utilize both glycolysis and oxidative phosphorylation to produce energy for its suppressive role due to the elevated metabolic state of the tumor-bearing host (40). Inhibition of two key enzymes in glycolysis: hexokinase (HK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by 2-deoxyglucose (2-DG) and sodium iodoacetate (IA), respectively, reduced MDSCs expansion, leading to a delay in tumor progression via induction of ROS-mediated apoptosis of MDSCs (40).

Another study reported that latent membrane protein 1 (LMP1) associated with Epstein-Barr virus mediates glycolysis by upregulating GLUT1 in tumor (41). This promotes the induction of GM-CSF, IL-6, and IL-1β production through COX-2 and NLRP3 inflammasome signaling pathways to enhance MDSCs differentiation and expansion, thereby promoting nasopharyngeal carcinoma (NPC) progression (41). However, a more recent study reported that EBV-encoded LMP1 induces *de novo* lipogenesis and lipid droplets formation through the activation of sterol regulatory element-binding protein 1 (SREBP1) which promotes progression of NPC (42). This suggests that LMP1 could also mediate other metabolic pathways such as lipogenesis (previously reported) or FAO to regulate MDSCs alteration in NPC progression. Therefore, a comprehensive study on the role of LMP1 expression in regulating immune cells (especially MDSC) in tumor state could help broaden understanding of the most upregulated pathway in MDSCs.

A recent study reported the correlation between MDSCs and glycolysis in human triple negative breast cancer (TNBC) and observed that restriction of glucose metabolism inhibits G-CSF and GM-CSF expression (43). This resulted in reduced MDSCs number while conferring tumor immunity by enhancing T-cell function. MDSCs are able to utilize anaerobic glycolysis when oxygen supply is limited to enhance their immunosuppressive role in the tumor microenvironment (44). This was observed by the upregulation of lactate dehydrogenase A (LDHA) (43), an enzyme involved in the reversible reaction of pyruvate to lactic acid. This could be an indicator of highly proliferative and energy demanding cell for the production of NAD^+ in subsequent ATP generation when oxidative phosphorylation is restricted due to insufficient oxygen availability. Inhibition of LDHA in a murine pancreatic cancer model decreased MDSCs frequency in the spleen and enhanced cytolytic activity of natural killer (NK) cells (44). Extrinsic lactic acid also increased the proportion of MDSCs derived from bone marrow (BM) cultured cells in the presence of GM-CSF and IL-6. Furthermore, MDSCs undergoing anaerobic glycolysis partly oxidize L-glutamine to provide a favorable condition for tumor growth (45). Although anaerobic glycolysis occurs 100 times faster than oxidative phosphorylation, it is less efficient and only helps in fulfilling a short-term energy requirement when oxygen supply is low (46).

Based on the diversity and dynamic attributes of the tumor milieu across various cancers as well as the stage of progression of same cancer, it is possible that the process of nutrient metabolism in immune cells might also differ across these conditions (39, 47). Recent studies have reported that the switch between glycolysis and oxidative phosphorylation in tumor-associated macrophages (TAM) is dependent on the stages of cancer development (48, 49). In relation to TAM, MDSCs also exhibit a certain degree of plasticity and may adopt a typically activated (M1) or alternatively activated (M2) phenotype, with antitumor or tumor-promoting roles, respectively (50). Therefore, the alterations of MDSCs differentiation, maturation and function may rely on overall central carbon metabolism and upregulation of cellular bioenergetics fluxes (45).

So far, the metabolic preference of MDSCs in tumor microenvironments is not fully known and requires more robust investigations. However, current evidence suggests that it may involve global regulation of metabolic flux.

OXIDIZED LIPIDS REGULATE MDSCS FUNCTION IN THE TUMOR MICROENVIRONMENT

Utilization of oxidized lipid as an energy source is crucial to the immunosuppressive roles of MDSCs in the tumor microenvironment (24). Gabrilovich et al. demonstrated that accumulation of oxidized lipids in tumor-infiltrating $CD11c^+$ DCs blocks antigen presentation and their orientation on major histocompatibility complex (MHC) class II (51, 52). This, in turn, blocks antigen-mediated cross-presentation and inhibits T cell stimulation. They also showed that targeting ACC1 with 5- (tetradecycloxy)-2-furoic acid (TOFA), reverses the effects of lipids, suggesting that the fatty acid biosynthesis pathway is involved in this process (**Figure 2**) (51).

In line with other myeloid cells, substantial lipid accumulation was observed in tumor-derived MDSCs (24, 53). MDSCs with lipid overload demonstrated greater immunosuppressive effect on $CD8^+$ T cells, compared to MDSCs with normal lipid content. Lipid accumulation in tumor-derived MDSCs can be linked to an increase in fatty acid uptake. This is supported by the study of Cao et al., which revealed an increased expression of fatty acid transport protein 4 (FATP4) in murine tumor-derived MDSCs (53). Most of the lipids detected in the MDSCs of tumor-bearing mice and cancer patients were found to be oxidized (**Figure 2**), possibly resulting from the oxidative activities of reactive oxygen species (ROS) and myeloperoxidase (MPO) (24, 54). Inhibition of ROS and MPO in these cells almost completely expunged the oxidation of lipids and resulted in MDSCs with a diminished immunosuppressive activity (24).

A recent study identified the upregulation of FATP2 on PMN-MDSC as a critical regulator of their immunosuppressive function (26). FATP2 promotes the accumulation of arachidonic acid leading to prostaglandin E2 (PGE2) synthesis in PMN-MDSCs thereby boosting their immunosuppressive activities. Thus, the pharmacological inhibition of FATP2 could serve as a novel and targeted therapeutic strategy to block the

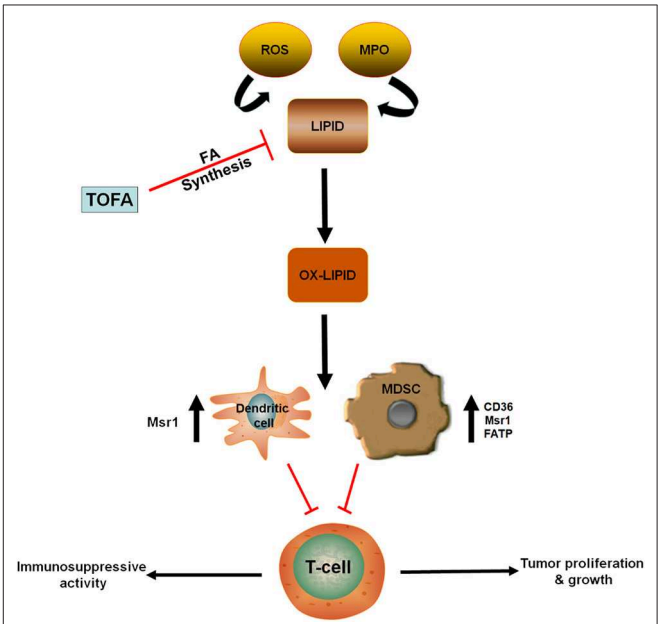

FIGURE 2 | Oxidized lipids contribute to the immunosuppressive role of MDSCs and DC. ROS and MPO contribute to the oxidation of lipid accumulated in antigen presenting cells (DC) and MDSCs. In these cells, upregulation of lipid transporters (CD36, Msr1, FATP) increase fatty acid uptake. Hence, promoting immunosuppressive activity and reducing T-cell function. However, treatment with TOFA (fatty acid synthesis inhibitor) blocked the accumulation of lipid in both DC and MDSCs. CD36, Cluster of differentiation 36; DC, Dendritic cell; FATP, Fatty acid transport protein; MDSCs, Myeloid-derived suppressor cells; MPO, Myeloperoxidase; Msr1, Macrophage scavenger receptor 1; Ox-lipid, Oxidized lipid; ROS, Reactive oxygen species; TOFA - 5, (tetradecycloxy)-2-furoic acid.

immunosuppressive activity of PMN-MDSCs. Collectively, these studies suggest the critical role of oxidized lipids in regulating myeloid cells function and specifically in MDSC as a potential therapeutic target in cancer.

EXOGENOUS FATTY ACID UPTAKE ENHANCES SUPPRESSIVE ACTIVITY IN MDSCS

MDSCs take up fatty acids from the tumor microenvironment and utilize them via several pathways. Our group previously reported that polyunsaturated fatty acids (PUFAs) impaired myeloid cell differentiation in bone marrow from tumor-bearing mice thus promoting the accumulation and functional activity of MDSCs (55). The study further demonstrated that dietary intake of linoleic acid (LA) and alpha-linolenic acid (ALA) promoted tumor growth in agreement with the observation by another group (56). It was recently discovered that culturing MSC-2, a myeloid suppressor cell line in the presence of long chain unsaturated fatty acids, oleate and linoleate increased lipid droplet accumulation which in-turn suppressed T-cell activity (57). However, T-cell activation remained unaffected in MSC-2 cells cultured in the presence of stearate, a saturated fatty acid that also accumulated lipid droplets. Removal of oleate from the culture medium triggered the mobilization of lipid droplets in this cell-line, thereby diminishing its immunosuppressive activity. Furthermore, it was observed that inhibiting diacylglycerol acyltransferases (DGAT) abolishes oleate-induced lipid droplet formation and impaired the immunosuppressive activity of MSC-2 (57). In addition, another group of researchers reported that MDSCs treated with linoleic acid demonstrated a stronger inhibitory effect on T-cell compared to those treated with palmitic acid which is a saturated fatty acid (53). In summary, unsaturated fatty acids which are known to be more susceptible to oxidation, contribute to the suppressive ability of MDSCs during cancer via upregulation of lipid metabolic gene such as DGAT.

SIGNALING PATHWAYS INVOLVED IN LIPID METABOLISM OF MDSCS

Recent findings suggest a relationship between oxidative phosphorylation initiated by lipid metabolism and its contribution to immunosuppressive myeloid cells. However, the underlying molecular mechanisms associated with FAO in subpopulations of tumor-infiltrating MDSCs is yet to be fully elucidated (54, 58). The role of kinases like AMPK and PI3K, transcription factors such as STATs, enzymes involved in FAO as well as several receptors including Peroxisome proliferator-activator receptors (PPARs), among others on immune cells, including MDSC, has been described in different *in vitro* and *in vivo* models. For example, pharmacological inhibition of STAT3 and STAT5 (activated by tumor-derived cytokines) decreased lipid accumulation, mitochondrial metabolism, and immunosuppressive function in MDSCs in an *in vitro* study (25). Increased expression of crucial genes encoding enzymes in FAO is linked to the suppressive role of tumor MDSCs which was abolished by FAO inhibitors (27). More detailed description of those critical components of the signaling pathways involved in the cellular lipid metabolism is described below.

LXR

Liver X receptors comprise two isoforms, LXRα and LXRβ which are encoded by *Nr1h3* and *Nr1h2*, respectively. Both isoforms are members of the nuclear hormone receptor family that modulate several transcriptional factors. LXR acts as a critical regulator of lipid homeostasis (**Figure 3**) by driving the expression of key genes involved in cholesterol, fatty acid, and glucose metabolism (59, 60).

Masoud et al., reported the effect of LXR activation on MDSCs expansion and their immunosuppressive activities on T-cell stimulation both *in vivo* and *in vitro* (61). RGX-104, an LXR agonist, significantly decreased the abundance of PMN-MDSCs and M-MDSCs from B16F10 melanoma tumor-bearing mice. The proportion of PMN-MDSCs generated *in vitro* from bone marrow cells treated with LXR agonist was decreased in the presence of GM-CSF. Oral administration of RGX-104 also decreased the population of MDSCs in cancer patients. It was previously reported that cholesterol-induced LXR sumoylation blocks IL-9 expression in $CD8^+$ T cells, partially by reducing the binding of NF-κB p65 subunit to IL-9 promoter (62). IL-9 demonstrates a critical role in the antitumor response of $CD8^+$ T cell subset (Tc9) (62–64). Also, IL-9 expressing T-cells have been identified in humans (65) and it was reported to enhance the function and survival of human tumor-infiltrating T-cells (66). It is imperative to decipher how the accumulation of lipids or exogenous fatty acid uptake regulates LXR signaling pathways in immune cells.

LXR promotes the transcriptional activation of a secretory protein, apolipoprotein E (ApoE) (61), which mediates the cellular uptake of lipoprotein particles by binding to low-density lipoprotein receptor (LDLR) and chylomicron remnants receptor (67). ApoE interaction with these receptors activates lipid (such as cholesterol, phospholipid, and triglycerides) metabolic pathways (68). A recent study showed that ApoE regulates MDSCs survival and tumor progression; $ApoE^{-/-}$ mice had increased levels of PMN-MDSCs and M-MDSCs. MDSCs from ApoE deficient mice showed reduced T-cell proliferation *in vitro* (61). Since ApoE plays a crucial role in lipoprotein metabolism (69), its deletion could enhance MDSCs immunosuppressive activity on T-cells via lipid accumulation. Al-Khami et al., reported the accumulation of lipid in bone-marrow derived MDSCs following extracellular uptake of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (25). This was observed to enhance oxidative metabolism and upregulation of ARG1 in MDSCs. Altogether, these suggest that the regulation of lipid metabolism via ApoE expression may alter MDSCs function in the tumor milieu.

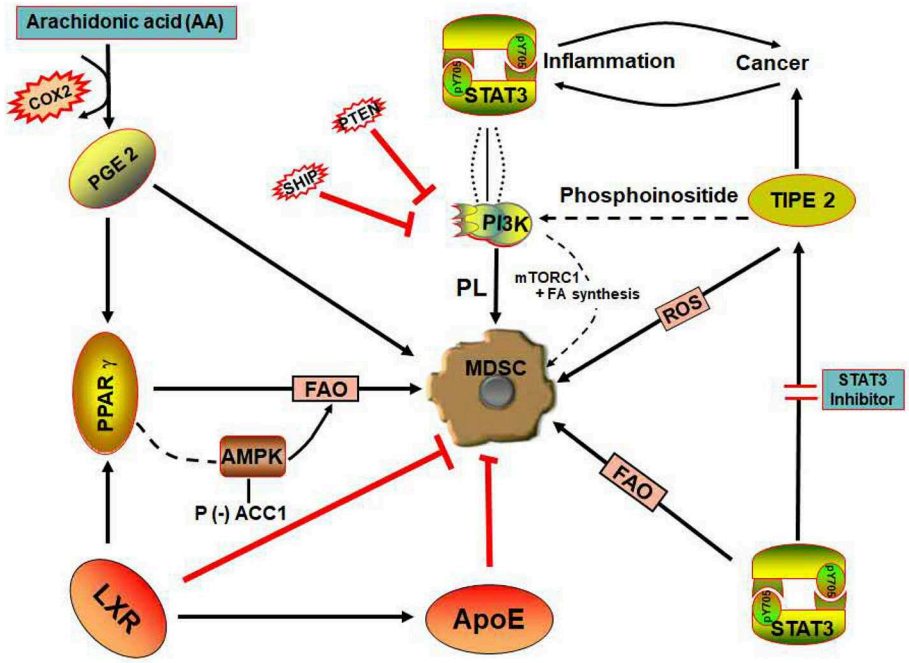

FIGURE 3 | Signaling pathways involved in lipid metabolism of MDSCs. (i) SHIP and PTEN are negative regulators of PI3K/AKT—involved in the promotion of lipid and sterol synthesis (ii) COX-2 is the enzyme which catalyzes arachidonic acid into PGE2, a pro-inflammatory lipid mediator that could result in elevated MDSCs. (iii) PPAR-γ initiates AMPK activation, thereby promoting FAO in MDSCs to enhance its immunosuppressive ability. (iv) LXR is a nuclear hormone receptor that regulates lipid homeostasis and enhances the transcriptional activation of ApoE—involved in lipoprotein metabolism. LXR inhibits MDSCs suppressive activity on T-cells. (v) STAT3 signaling enhances FAO and also upregulates TIPE2 expression in MDSCs. (vi) TIPE2, a promoter of the immunosuppressive function of MDSCs, regulates PI3K via signaling of phosphoinositide and can be inhibited by STAT3 inhibitors. ACC1, Acetyl CoA carboxylase 1; AMPK, AMP-activated protein kinase; APOE, Apolipoprotein E; COX-2, Cycloxygenase 2; FAO, Fatty acid oxidation; LXR, Liver X receptors; MDSCs, Myeloid-derived suppressor cells; mTORC1, mammalian target of rapamycin complex 1; PGE2, Prostaglandin E2; PI3K, Phosphoinositide-3-Kinase; PL, Phospholipid; PPARγ, Peroxisome proliferator-activator receptors gamma; PTEN, Phosphatase and tensin; ROS, Reactive oxygen species; SHIP—5-inositol phosphatase; STAT 3, Signal transducer and activator of transcription; TIPE 2, Tumor necrosis factor alpha-induced protein 8 like 2; P, Phosphorylation; +, Stimulate; –, Deactivation.

PPARs

Peroxisome proliferator-activator receptors (PPARs) are "lipid sensing" nuclear receptors activated by free fatty acid (FFA), prostaglandins, eicosanoids, or sterols (70). They are divided into three subtypes which are: PPARα, PPARγ, and PPARδ. PPARγ and PPARδ elicit the expression of certain FAO genes (**Figure 3**) and coordinate anti-inflammatory functions while PPARα directly upregulates the expression of CPT1a (71), a crucial enzyme involved in mitochondrial fatty acid oxidation.

PPARγ plays an important role in regulating lysosomal acid lipase (LAL) activity, a key enzyme in the metabolism of neutral lipids. $LAL^{-/-}$ MDSCs demonstrate greater immunosuppression thereby promoting tumor cell proliferation, growth and metastasis. Activation of PPARγ pathway in $LAL^{-/-}$ MDSCs impaired tumor growth and metastasis *in vivo* as well as *in vitro* (72). Cardiolipin, a phospholipid, promotes IL-10 expression in MDSCs from the lungs of tumor-bearing mice by activation of PPARγ activity (70, 73) but this can be inhibited by GW9662 (a specific inhibitor of PPARγ) (74). However, the roles of the PPAR family in tumor biology remain unclear.

PPARα was reported to mediate the transcription initiation of CPT gene in $CD4^+$ T-cells isolated from Jurkat cell and a murine model of non-alcoholic fatty liver disease (NAFLD, a risk factor of hepatocellular carcinoma) (75) treated with linoleic acid. PPARα agonist, benzofibrate, increased the generation of mitochondrial ROS and induced apoptosis by upregulating CPT1a following treatment with LA in murine and Jurkat cell. This effect was reversed in the presence of PPARα inhibitor (GW6471), illuminating the important role PPARα may play in regulating CPT1 (76).

Furthermore, PPARα agonist, fenofibrate, enhanced fatty acid catabolism in $CD8^+$ T-cell under hypoxia and low glucose condition (77) thereby activating genes encoding proteins (such as PPARα and CPT1a) involved in lipid metabolism and TCA cycle. Vaccinated animals to elicit melanoma-specific $CD8^+$ TILs response and treated with fenofibrate significantly delayed tumor progression, confirming that enhanced fatty acid catabolism improves $CD8^+$ TILs functions. In PPARα KO mice, $CD8^+$ cells cultured in glucose-deprived media exhibited a reduction in the transcript for fatty acid metabolism and lower functionality in comparison with the wild-type cells (77). This study suggests fatty acid catabolism is essential for $CD8^+$ TILs functions when access

to glucose is limited. Therefore, targeting PPARs pathways could be another promising option in manipulating lipid content in MDSCs for successful cancer therapy.

AMPK

AMP-activated protein kinase (AMPK) is a potential mediator of lipid metabolism regulating cellular homeostasis in MDSCs (45). Activation of PGC1β/PPARγ axis induces AMPK signaling, demonstrating a crucial role in mitochondrial biogenesis; thereby activating the expression of genes encoding proteins involved in FAO (15). More so, AMPK regulates the phosphorylation and deactivation of ACC1, the key enzyme involved in the regulation of fatty acid synthesis (78) (**Figure 3**).

MDSC-mediated AMPK phosphorylation could increase survival of multiple myeloma cells. Treatment with compound C (AMPK inhibitor) in the presence or absence of MDSCs decreased AMPK phosphorylation and induced apoptosis of multiple myeloma cells (79). Oxidative stress and upregulation of HIF-1α were reported as triggers of AMPK activation in osteosarcoma cells (80, 81). Since MDSCs from tumor-bearing mice and cancer patients demonstrate a high amount of intracellular ROS (40, 82–84), it is possible that accumulated ROS may enhance AMPK activity. In addition, cytokines such as IL-10 and TGF-β are elevated in MDSCs (85, 86) and may also induce AMPK phosphorylation.

Although the role of AMPK activation in cancer is widely studied and considered as a tumor suppressor [reviewed in (87)], its activity in modulating MDSCs function remains unclear. There have been conflicting reports on the exact role of AMPK in modulating MDSCs activity. It was recently demonstrated that pharmacological targeting of AMPK abrogates MDSCs function in tumors by repressing the expression of iNOS, arginase, and promoting T-cell proliferation (88, 89). Another report suggested that AMPK signaling enhances MDSCs immunosuppressive activity in doxorubicin-resistant tumors via upregulation of miR-10a expression (90). However, inhibition of miR-10a abrogated the elevated $CD11b^{+}Gr1^{+}$ MDSCs frequency and also M2 signature genes such as ARG1, TGF-β, and MMP9. Hence, there is a need for more comprehensive studies to elucidate the role of this potential lipid metabolic mediator on MDSCs function in the tumor microenvironment.

PI3K/AKT/mTOR

Phosphoinositide-3-Kinase (PI3K) signaling performs an important role in regulating cellular functions and coordinate processes such as protein synthesis, glucose homeostasis, cellular metabolism, cell growth, migration, and survival (91). PI3K catalyzes the phosphorylation of phosphatidylinositol in the plasma membrane by adding a phosphate moiety to the 3'OH on the lipid (92). Several scientists investigating the etiology of cancer at the molecular level have intensely studied this pathway due to its frequent alteration in cancer (93). PI3K has been reported to regulate physiological activities in neutrophils (94, 95). In aging mice, bone marrow and secondary lymphoid organs accumulate a substantial level of MDSCs, which may be associated with altered PI3K/AKT signaling pathway. The molecular mechanism enhancing the suppressive activity of MDSCs revealed an upregulation in iNOS activity and inhibition of T-cell activation. This leads to an alteration in the immune system and supports immune senescence (96). These observations made PI3K signaling pathway a novel target for new cancer therapy (97) and we consider its signaling may be a potential modulator of lipid accumulation in MDSCs to enhance its suppressive activity on T-cells proliferation.

Phosphatase and tensin (PTEN) and 5-inositol phosphatase (SHIP) are negative regulators of the PI3K/AKT signaling pathway (**Figure 3**) regulating phosphoinositide metabolism in immune cells (98). SHIP suppresses cell growth and survival via the movement of cell membranes (shortly after stimulation of the extracellular compartment) leading to the conversion of phosphatidylinositol-3,4,5-triphosphate (PIP3) into phosphatidylinositol-3, 4- bisphosphate (PI3,4-P2), thus inhibiting PI3K (99). The MDSCs population can be increased through the downregulation of SHIP expression by cancer cells secreting factors such as GM-CSF, IL-6, and TGF-β. It was previously reported that increased MDSCs population in $SHIP^{-/-}$ tumor-bearing mice lymphoid compartment contributes to immunosuppressive allogenic T-cell responses *in vitro* and *in vivo* (100). Hence, treatment strategies toward enhancing the activity of SHIP could mitigate the immunosuppressive effect of MDSCs and serve as therapeutic approaches in cancer (101).

The mammalian target of rapamycin (mTOR) is a major component of the PI3K/AKT pathway involved in cell proliferation and nutrient availability. It also controls the innate and adaptive immune response in multiple immune cells (102). Inhibition of mTOR signaling with rapamycin-induced the upregulation of arginase-1 and iNOS in MDSCs (103). Thus, enhancing MDSCs immunosuppressive activity and reduced T-cells proliferation. Constitutive initiation of Akt has been documented to promote lipid and sterol synthesis in addition to glycolysis (104, 105). Given that genetic manipulation of mTORC1 (either by deletion of TSC1 or TSC2 in fibroblast) activate downstream transcription factors involved in lipid biosynthesis (106), the signaling pathway via Akt-TSC-mTORC1-S6K1-SREBP axis could be a possible pathway in MDSC. When mTORC1 is constitutively active, the transcription factor, SREBP is activated and drives the expression of sterol and fatty acid biosynthesis genes (106). The role of mTORC2 in lipid regulation unlike mTORC1 still remains unclear. Chen et al., recently highlighted another mTORC2 target, ATP citrate lyase (ACLY) in lipid metabolism. It converts citrate derived from the TCA cycle into acetyl CoA in the cytoplasm where it can be used for lipid biosynthesis. ACLY was identified as a target of mTORC2 in a breast cancer cell line; revealing that mTORC2 and not mTORC1 is necessary for the generation of acetyl CoA in an ACLY-catalyzed reaction (107). Inhibition of ACLY or mTORC2 activity altered mitochondrial function by reducing cell proliferation and tumor growth (108). Since mTORC2 can regulate lipid metabolism by limiting the activity of ACLY to generate the building blocks of lipids, targeting this signaling pathway could attenuate the immunosuppressive activities of MDSCs.

Increased mRNA and protein synthesis resulting from the phosphorylation of S6K and 4EBP1 proteins via mTOR enhanced cell proliferation (109). Reports had shown the possible regulatory role of the mTOR pathway during inflammatory responses by an alteration in the activity of STAT3 and NF-κB in myeloid cells (110). Chen et al., showed the engagement of the mTOR signaling pathway in monocytes differentiation to TAM (111). The effect of mTOR signaling in promoting the expression of lipid and sterol genes when they are activated during myelopoiesis could contribute to lipid accumulation in MDSCs thereby enhancing their immunosuppressive function. Despite the progress made in studying this signaling pathway, how mTOR affects the immunosuppressive function of MDSCs is yet to be substantiated.

STAT

Members of the signal transducer and activator of transcription (STAT) protein family has been reported to regulate MDSCs functions by coordinating various activities (112). STAT3 signaling is crucial for the activation and expansion of MDSCs in several pathophysiological conditions (113) (**Figure 3**). STAT3 and STAT5 signaling induced by G-CSF and GM-CSF, respectively, regulates the expression of proteins critical for expansion, differentiation and activation of MDSC (32). Recently, Al-Khami et al., demonstrated that the pharmacological blockage of STAT3 by FLLL32 or STAT5 by pimozide in BM-derived MDSCs decreased the level of intracellular accumulated neutral lipids. More importantly, these inhibitors prevented the induction of arginase-1 and iNOS, thereby abrogating the development of immunosuppressive functions of MDSCs. The effects of STAT3 and STAT5 inhibition suggest the role of lipids in driving the immunosuppressive function of MDSCs (25). Despite the available evidence, there is still a gap in understanding how STAT3/STAT5 signaling regulates lipid metabolism and immunosuppressive mechanisms in MDSCs.

Our group previously discovered that culturing mouse bone marrow-derived MDSCs in the presence of LA, elevated MDSCs proliferation while co-treatment with JS1-124 (STAT3 inhibitor) reversed this effect. MDSCs generated *in vitro* with or without JSI-124 or LA treatment was co-cultured with allogenic T-cells for 3 days to evaluate the influence of STAT3 on MDSCs suppressive activity by CFSE dilution. The proliferation of T-cell was elevated in the co-treated group compared to LA treated group (55). A marked decrease was observed in the suppressive ability of MDSCs generated in the presence of LA and JS1-124-treated cells (55). Furthermore, STAT3 inhibition or blockage in the expression of STAT3 in conditional knockout mice led to a decline in MDSCs number and improved T-cell response in tumor (114).

The transcriptional factor STAT5 can be activated by GM-CSF which has a key role in myelopoiesis and expansion of MDSCs (115). It was recently reported that GM-CSF in PMNs control the expression of FATP2 through the phosphorylation of STAT5 (26). Deletion of STAT5 in PMNs slowed tumor growth in comparison with the control mice. This was associated with a decreased expression of FATP2 in PMNs. Hence, STAT family could be another potential target for the immunosuppressive activity of MDSCs via fatty acid regulation.

TIPE Family

Tumor necrosis factor alpha-induced protein 8 like (TIPE or TNFAIP8L) family is a group of recently established regulators of tumorigenesis and immunity (116, 117). There are four homologous mammalian members of this family that have been identified including TIPE (the primary member of the family), TIPE1, TIPE2, and TIPE3 (118). TIPE and TIPE1 are ubiquitously expressed members, TIPE2 is found in the hematopoietic cells and TIPE3 expression is restricted to secretory epithelial tissues.

TIPE family was reported as the only defined transfer protein of second messenger molecules, phosphatidylinositol 4,5-bisphosphate (PIP2) and PIP3 (119, 120). Research revealed the involvement of TIPE family in the transport of phospholipids in and out of the plasma membrane via signaling of phosphoinositide to regulate PI3K (119) (**Figure 3**). Activation of PI3K can also stimulate the STAT3 pathway which is related to cancer and inflammation (121–123) as well as the NF-κB signaling (124, 125). These signaling pathways play important roles in lipid metabolism and contribute to the immunosuppressive activities of MDSCs (25, 55, 100, 101).

TIPE2 expression is most abundant in the hematopoietic cells (including MDSCs) and T-cells. Upon lipopolysaccharide (LPS) stimulation, nitric oxide production was increased due to loss of TIPE2 gene in macrophages (126). Likewise, it modulates macrophage response to oxidized low-density lipoproteins (ox-LDL). Its deficiency in macrophage enhanced the stimulation of inflammatory cytokines which induced oxidative stress associated with p38, NF-κB, and JNK signaling (127). In relation to these findings, TIPE2 deficient bone marrow increased the formation of atherosclerosis in $Ldlr^{-/-}$ mice consuming ox-LDL and high-fat diet, suppressed TIPE2 mRNA expression (127). Exploring the crosstalk of lipid metabolism in MDSCs with TIPE2 could be another promising approach in cancer therapy.

PGE2

PGE2 is a pro-inflammatory molecule elicited by stromal, cancer, infiltrating myeloid cells, and associated with G-protein-coupling receptors (GPCRs). PGE2 is an important bioactive lipid and active product of cyclooxygenase 2 (COX-2). COX-2 catalyzes metabolic pathway involving the transformation of AA to an unstable intermediate PGG2, then to endoperoxide H2 (PGH2) and later into five primary prostanoids (TXA2, PGD2, PGE2, PGF2α, and PGI2) through cell-specific synthase (128). In MDSCs, PGE2 signals via the PGE2 receptor, E-prostanoid 4 (EP4) and upregulates arginase 1 activity in this cell, thereby enhancing its immunosuppressive role (129, 130).

Production of COX-2 increased MDSCs proliferation (**Figure 3**) correlated to an upregulation in the expression of arginase-1 and iNOS in murine tumor-infiltrating leukocytes (131), thereby promoting tumor. Excess COX-2 stimulated the proliferation of malignant cells, thus compromised tumor immunity (132). PGE2/COX-2 signaling was involved in the differentiation of DC into MDSCs in an *in vitro* study (133). This

impaired DC maturation and its antigen presentation ability, thus inhibiting MHC class II expression and T cell activation (134). Exploring COX-2 expression as a potential target could be a means to enhance immune surveillance and cancer therapy.

Studies on renal carcinoma cells (RCC) revealed the immunosuppressive influence of PGE2 on tumor cells through induction of arginase activity in MDSCs (135). This hindered T-cell activity in the tumor microenvironment resulting from the unavailability of L-arginine (136). Therefore, manipulation of PGE2 expression in MDSCs could enhance immunotherapy (137).

CXCR4 expression in differentiating MDSCs was elevated by the involvement of PGE2 in mice tumor cells (138, 139). Obermajer et al., established in human ovarian cancer that tumor-related PGE2, induced CXCL12 chemokine production and the expression of CXCR4 on MDSCs. The study also reported that PGE2 promotes COX2 expression in MDSCs. However, exposure of MDSCs from ovarian cancer cells to COX2 inhibitors decreased CXCR4 expression and sensitivity to recombinant CXCL12 (140). PGE2 possibly drives MDSCs accumulation by mobilizing chemokines to attract them from the circulation into the tumor microenvironment. COX-2 selective inhibitors and conventional non-steroidal anti-inflammatory drugs (NSAIDS) have previously been established to suppress immune evasion in tumors. It has been proposed that COX-2 inhibitors may stimulate type 1 immune responses by inhibiting MDSCs function (141).

A recent study reported that FATP2 promoted the suppressive role on PMN-MDSCs through the synthesis of PGE2, following exogenous uptake of AA (26). This suggests that regulation of the metabolic pathway involved in the transformation of AA to PGE2 via COX-2 may be a promising path in controlling lipid accumulation and attenuate the immunosuppressive function of MDSCs.

CONCLUSIONS

Metabolic alteration in cancerous cells has long been reported, however, a salient question yet to be fully investigated is the metabolic fate of tumor-associated immune cells. A better understanding of immunosuppression from a metabolic perspective may enhance the identification of new immunotherapeutic targets (142). Lipid metabolic reprogramming of MDSCs is a major contributing factor to its altered phenotype and co-opted immunosuppressive function. In MDSCs, the factors regulating the shift from glycolysis to FAO-OXPHOS in the tumor milieu and the molecular or transcriptional networks controlling its immunosuppressive role have not been fully explored. Understanding the precise roles of different forms of lipids in the tumor microenvironment is challenging. More research focus on elucidating lipid metabolism in MDSCs may enhance the development of therapies to treat cancer in the clinic.

AUTHOR CONTRIBUTIONS

DY, AA, and XW developed the study and wrote the paper. MX, LA, GZ, and YC contributed to the critical suggestion.

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REFERENCES

1. Bennett JA, Rao VS, Mitchell MS. Systemic bacillus Calmette-Guerin (BCG) activates natural suppressor cells. *Proc. Natl. Acad. Sci USA.* (1978) 75:5142–4. doi: 10.1073/pnas.75.10.5142
2. He YM, Li X, Perego M, Nefedova Y, Kossenkov AV, Jensen EA, et al. Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation. *Nat Med.* (2018) 24:224–31. doi: 10.1038/nm.4467
3. Gabrilovich DI, Bronte V, Chen S-H, Colombo MP, Ochoa A, Ostrand-Rosenberg S, et al. The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* (2007) 67:425. doi: 10.1158/0008-5472.CAN-06-3037
4. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* (2016) 7:12150. doi: 10.1038/ncomms12150
5. De Sanctis F, Solito S, Ugel S, Molon B, Bronte V, Marigo I. MDSCs in cancer: Conceiving new prognostic and therapeutic targets. *Biochim Biophys Acta.* (2016) 1865:35–48. doi: 10.1016/j.bbcan.2015.08.001
6. Amodio G, Cichy J, Conde P, Matteoli G, Moreau A, Ochando J, et al. Role of myeloid regulatory cells (MRCs) in maintaining tissue homeostasis and promoting tolerance in autoimmunity, inflammatory disease and transplantation. *Cancer Immunol Immunother.* (2018) 68:661–72. doi: 10.1007/s00262-018-2264-3
7. Zoeller M, Zhao K, Kutlu N, Bauer N, Provaznik J, Hackert T, et al. Immunoregulatory effects of myeloid-derived suppressor cell exosomes in mouse model of autoimmune alopecia areata. *Front Immunol.* (2018) 9:1279. doi: 10.3389/fimmu.2018.01279

8. Li M, Zhu D, Tian J, Wang S. Roles of myeloid-derived suppressor cell subpopulations in autoimmune arthritis. *Front Immunol.* (2018) 9:2849. doi: 10.3389/fimmu.2018.02849
9. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest.* (2015) 125:3356–64. doi: 10.1172/JCI80005
10. Peranzoni E, Zilio S, Marigo I, Dolcetti L, Zanovello P, Mandruzzato S, et al. Myeloid-derived suppressor cell heterogeneity and subset definition. *Curr Opin Immunol.* (2010) 22:238–44. doi: 10.1016/j.coi.2010.01.021
11. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol.* (2008) 181:5791–802. doi: 10.4049/jimmunol.181.8.5791
12. Youn JI, Gabrilovich DI. The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. *Eur J Immunol.* (2010) 40:2969–75. doi: 10.1002/eji.201040895.
13. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol Immunother.* (2012) 61:1155–67. doi: 10.1007/s00262-012-1294-5
14. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann N Y Acad Sci.* (2014) 1319:47–65. doi: 10.1111/nyas.12469
15. Mohamed E, Al-Khami AA, Rodriguez PC. The cellular metabolic landscape in the tumor milieu regulates the activity of myeloid infiltrates. *Cell Mol Immunol.* (2018) 15:421. doi: 10.1038/s41423-018-0001-7
16. Porta C, Sica A, Riboldi E. Tumor-associated myeloid cells: new understandings on their metabolic regulation and their influence in cancer immunotherapy. *FEBS J.* (2018) 285:717–33. doi: 10.1111/febs.14288
17. Olenchock BA, Rathmell JC, Vander Heiden MG. Biochemical underpinnings of immune cell metabolic phenotypes. *Immunity.* (2017) 46:703–13. doi: 10.1016/j.immuni.2017.04.013
18. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol.* (2017) 38:395–406. doi: 10.1016/j.it.2017.03.001
19. Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T cell metabolism in health and disease. *Nat Rev Immunol.* (2018) 18:19–34. doi: 10.1038/nri.2017.99
20. Davies LC, Rice CM, McVicar DW, Weiss JM. Diversity and environmental adaptation of phagocytic cell metabolism. *J Leukoc Biol.* (2018) 105:37–48. doi: 10.1002/JLB.4RI0518-195R
21. Den Brok MH, Raaijmakers TK, Collado-Camps E, Adema GJ. Lipid droplets as immune modulators in myeloid cells. *Trends Immunol.* (2018) 39:380–92. doi: 10.1016/j.it.2018.01.012
22. Al-Khami AA, Rodriguez PC, Ochoa AC. Metabolic reprogramming of myeloid-derived suppressor cells (MDSC) in cancer. *Oncoimmunology.* (2016) 5:e1200771. doi: 10.1080/2162402X.2016.1200771
23. Clements VK, Long T, Long R, Figley C, Smith D, Ostrand-Rosenberg S. Frontline science: high fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. *J Leukoc Biol.* (2018) 103:395–407. doi: 10.1002/JLB.4HI0517-210R
24. Veglia F, Tyurin V, Kagan V, Gabrilovich D. Oxidized lipids contribute to the suppression function of myeloid derived suppressor cells in cancer. In: *Proceedings of the 106th Annual Meeting of the American Association for Cancer Research*. Philadelphia, PA: AACR (2015). doi: 10.1158/1538-7445.AM2015-467
25. Al-Khami AA, Zheng L, Del Valle L, Hossain F, Wyczechowska D, Zabaleta J, et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. *Oncoimmunology.* (2017) 6:e1344804. doi: 10.1080/2162402X.2017.1344804
26. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature.* (2019) 569:73–8. doi: 10.1038/s41586-019-1118-2
27. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *Cancer Immunol Res.* (2015) 3:1236–47. doi: 10.1158/2326-6066.CIR-15-0036
28. Alrob OA, Lopaschuk GD. Role of CoA and acetyl-CoA in regulating cardiac fatty acid and glucose oxidation. *Biochem Soc T.* (2014) 42:1043–51. doi: 10.1042/BST20140094
29. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol.* (2015) 36:81–91. doi: 10.1016/j.it.2014.12.005
30. Ganeshan K, Chawla A. Metabolic regulation of immune responses. *Annu Rev Immunol.* (2014) 32:609–34. doi: 10.1146/annurev-immunol-032713-120236
31. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol.* (2016) 16:553–65. doi: 10.1038/nri.2016.70
32. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* (2012) 12:253–68. doi: 10.1038/nri3175
33. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res.* (2004) 64:5839–49. doi: 10.1158/0008-5472.CAN-04-0465
34. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res.* (2010) 70:68–77. doi: 10.1158/0008-5472.CAN-09-2587
35. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med.* (1999) 189:1363–72. doi: 10.1084/jem.189.9.1363
36. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol.* (2006) 177:7303–11. doi: 10.4049/jimmunol.177.10.7303
37. Saxena V, Ondr JK, Magnusen AF, Munn DH, Katz JD. The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the nonobese diabetic mouse. *J Immunol.* (2007) 179:5041–53. doi: 10.4049/jimmunol.179.8.5041
38. Grohmann U, Bronte V. Control of immune response by amino acid metabolism. *Immunol Rev.* (2010) 236:243–64. doi: 10.1111/j.1600-065X.2010.00915.x
39. Sica A, Strauss L. Energy metabolism drives myeloid-derived suppressor cell differ -entiation and functions in pathology. *J Leukoc Biol.* (2017) 102:325–34. doi: 10.1189/jlb.4MR1116-476R
40. Jian SL, Chen WW, Su YC, Su YW, Chuang TH, Hsu SC, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. *Cell Death Dis.* (2017) 8:e2779. doi: 10.1038/cddis.2017.192
41. Cai TT, Ye SB, Liu YN, He J, Chen QY, Mai HQ, et al. LMP1-mediated glycolysis induces myeloid-derived suppressor cell expansion in nasopharyngeal carcinoma. *PLoS Pathog.* (2017) 13:e1006503. doi: 10.1371/journal.ppat.1006503
42. Lo AKF, Lung RWM, Dawson CW, Young LS, Ko CW, Yeung WW, et al. Activation of sterol regulatory element-binding protein 1 (SREBP1)-mediated lipogenesis by the Epstein–Barr virus-encoded latent membrane protein 1 (LMP1) promotes cell proliferation and progression of nasopharyngeal carcinoma. *J Pathol.* (2018) 246:180–90. doi: 10.1002/path.5130
43. Li W, Tanikawa T, Kryczek I, Xia H, Li G, Wu K, et al. Aerobic glycolysis controls myeloid-derived suppressor cells and tumor immunity via a specific CEBPB isoform in triple-negative breast cancer. *Cell Metab.* (2018) 28:87–103.e6. doi: 10.1016/j.cmet.2018.04.022
44. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol.* (2013) 191:1486–95. doi: 10.4049/jimmunol.1202702
45. Hammami I, Chen J, Murschel F, Bronte V, De Crescenzo G, Jolicoeur M. Immunosuppressive activity enhances central carbon metabolism and bioenergetics in myeloid-derived suppressor cells *in vitro* models. *BMC Cell Biol.* (2012) 13:18. doi: 10.1186/1471-2121-13-18
46. Valvona CJ, Fillmore HL, Nunn PB, Pilkington GJ. The regulation and function of lactate dehydrogenase a: therapeutic potential in brain tumor. *Brain Pathol.* (2016) 26:3–17. doi: 10.1111/bpa.12299

47. Biswas SK. Metabolic reprogramming of immune cells in cancer progression. *Immunity*. (2015) 43:435–49. doi: 10.1016/j.immuni.2015.09.001
48. Blagih J, Jones RG. Polarizing macrophages through reprogramming of glucose metabolism. *Cell Metab*. (2012) 15:793–5. doi: 10.1016/j.cmet.2012.05.008
49. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol*. (2010) 22:231–7. doi: 10.1016/j.coi.2010.01.009
50. Yang WC, Ma G, Chen SH, Pan PY. Polarization and reprogramming of myeloid-derived suppressor cells. *J Mol Cell Biol*. (2013) 5:207–9. doi: 10.1093/jmcb/mjt009
51. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, et al. Lipid accumulation and dendritic cell dysfunction in cancer. *Nat Med*. (2010) 16:880–6. doi: 10.1038/nm.2172
52. Cao W, Ramakrishnan R, Tuyrin VA, Veglia F, Condamine T, Amoscato A, et al. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. *J Immunol*. (2014) 192:2920–31. doi: 10.4049/jimmunol.1302801
53. Cao W, Gabrilovich D. Contribution of fatty acid accumulation to myeloid-derived suppressor cell function in cancer. In: *Proceedings of the 102nd Annual Meeting of the American Association for Cancer Research*. Orlando, FL; Philadelphia, PA: AACR (2011). doi: 10.1158/1538-7445.AM2011-3649
54. Veglia F, Tyurin VA, Kagan VE, Gabrilovich D. Lipids and suppressive functions of MDSC in cancer. In: *Proceedings of the Annual Meeting 2018*. Chicago, IL; Philadelphia, PA: AACR (2018).
55. Yan D, Yang Q, Shi M, Zhong L, Wu C, Meng T, et al. Polyunsaturated fatty acids promote the expansion of myeloid-derived suppressor cells by activating the JAK/STAT3 pathway. *Eur J Immunol*. (2013) 43:2943–55. doi: 10.1002/eji.201343472
56. Rose DP. Effects of dietary fatty acids on breast and prostate cancers: evidence from *in vitro* experiments and animal studies. *Am J Clin Nutr*. (1997) 66:1513S—22. doi: 10.1093/ajcn/66.6.1513S
57. Wu H, Weidinger C, Schmidt F, Keye J, Friedrich M, Yerinde C, et al. Oleate but not stearate induces the regulatory phenotype of myeloid suppressor cells. *Sci Rep*. (2017) 7:7498. doi: 10.1038/s41598-017-07685-9
58. Al-Khami AA, Rodriguez PC, Ochoa AC. Energy metabolic pathways control the fate and function of myeloid immune cells. *J Leukoc Biol*. (2017) 102:369–80. doi: 10.1189/jlb.1VMR1216-535R
59. Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat Rev Drug Discov*. (2014) 13:433–44. doi: 10.1038/nrd4280
60. Evans RM, Mangelsdorf DJ. Nuclear receptors, RXR, and the big bang. *Cell*. (2014) 157:255–66. doi: 10.1016/j.cell.2014.03.012
61. Tavazoie MF, Pollack I, Tanqueco R, Ostendorf BN, Reis BS, Gonsalves FC, et al. LXR/ApoE activation restricts innate immune suppression in cancer. *Cell*. (2018) 172:825–40.e18. doi: 10.1016/j.cell.2017.12.026
62. Ma X, Bi E, Huang C, Lu Y, Xue G, Guo X, et al. Cholesterol negatively regulates IL-9-producing CD8(+) T cell differentiation and antitumor activity. *J Exp Med*. (2018) 215:1555–69. doi: 10.1084/jem.20171576
63. Lu Y, Hong S, Li H, Park J, Hong B, Wang L, et al. Th9 cells promote antitumor immune responses *in vivo*. *J Clin Invest*. (2012) 122:4160–71. doi: 10.1172/JCI65459
64. Lu Y, Hong B, Li H, Zheng Y, Zhang M, Wang S, et al. Tumor-specific IL-9-producing CD8+ Tc9 cells are superior effector than type-I cytotoxic Tc1 cells for adoptive immunotherapy of cancers. *Proc Natl Acad Sci USA*. (2014) 111:2265–70. doi: 10.1073/pnas.1317431111
65. Schlapbach C, Gehad A, Yang C, Watanabe R, Guenova E, Teague JE, et al. Human TH9 cells are skin-tropic and have autocrine and paracrine proinflammatory capacity. *Sci Transl Med*. (2014) 6:219ra8. doi: 10.1126/scitranslmed.3007828
66. Parrot T, Allard M, Oger R, Benlalam H, Raingeard de la Bletiere D, Coutolleau A, et al. IL-9 promotes the survival and function of human melanoma-infiltrating CD4(+) CD8(+) double-positive T cells. *Eur J Immunol*. (2016) 46:1770–82. doi: 10.1002/eji.201546061
67. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, et al. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc Natl Acad Sci USA*. (2001) 98:507–12. doi: 10.1073/pnas.021488798
68. Huang Y, Mahley RW. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. *Neurobiol Dis*. (2014) 72(Pt A):3–12. doi: 10.1016/j.nbd.2014.08.025
69. El RA, Bard JM, Valin S, Huvelin JM, Nazih H. Macrophage apolipoprotein E and proliferation of MCF-7 breast cancer cells: role of LXR. *Anticancer Res*. (2013) 33:3783–9. doi : 10.1007/s11839-013-0432-4
70. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol Metab*. (2012) 23:351–63. doi: 10.1016/j.tem.2012.05.001
71. Song S, Attia RR, Connaughton S, Niesen MI, Ness GC, Elam MB, et al. Peroxisome proliferator activated receptor α (PPARα) and PPAR gamma coactivator (PGC-1α) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements. *Mol Cell Endocrinol*. (2010) 325:54–63. doi: 10.1016/j.mce.2010.05.019
72. Zhao T, Du H, Blum JS, Yan C. Critical role of PPARγ in myeloid-derived suppressor cell-stimulated cancer cell proliferation and metastasis. *Oncotarget*. (2016) 7:1529–43. doi: 10.18632/oncotarget.6414.
73. La Cour Poulsen L, Siersbæk M, Mandrup S. PPARs: fatty acid sensors controlling metabolism. *Semin Cell Dev Biol*. (2012) 23:631–9. doi: 10.1016/j.semcdb.2012.01.003
74. Chakraborty K, Raundhal M, Chen BB, Morse C, Tyurina YY, Khare A, et al. The mito-DAMP cardiolipin blocks IL-10 production causing persistent inflammation during bacterial pneumonia. *Nat Commun*. (2017) 8:13944. doi: 10.1038/ncomms13944
75. Llovet JM, Villanueva A. Liver cancer: effect of HCV clearance with direct-acting antiviral agents on HCC. *Nat Rev Gastroenterol Hepatol*. (2016) 13:561–2. doi: 10.1038/nrgastro.2016.140
76. Brown ZJ, Fu Q, Ma C, Kruhlak M, Zhang H, Luo J, et al. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4+ T cell apoptosis promoting HCC development. *Cell Death Dis*. (2018) 9:620. doi: 10.1038/s41419-018-0687-6
77. Zhang Y, Kurupati R, Liu L, Zhou XY, Zhang G, Hudaihed A, et al. Enhancing CD8+ T cell fatty acid catabolism within a metabolically challenging tumor micro- environment increases the efficacy of melanoma immunotherapy. *Cancer Cell*. (2017) 32:377–91.e9. doi: 10.1016/j.ccell.2017.08.004
78. Galic S, Fullerton MD, Schertzer JD, Sikkema S, Marcinko K, Walkley CR, et al. Hematopoietic AMPK β1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. *J Clin Invest*. (2011) 121:4903–15. doi: 10.1172/JCI58577
79. De Veirman K, Menu E, Maes K, De Beule N, De Smedt E, Maes A, et al. Myeloid-derived suppresor cells induce multiple myeloma cell survival by activating the AMPK pathway. *Cancer Lett*. (2018) 442:233–41. doi: 10.1016/j.canlet.2018.11.002
80. Zhao C, Zhang Q, Yu T, Sun S, Wang W, Liu G. Hypoxia promotes drug resistance in osteosarcoma cells via activating AMP-activated protein kinase (AMPK) signaling. *J Bone Oncol*. (2016) 5:22–9. doi: 10.1016/j.jbo.2016.01.002
81. Zhao Y, Hu X, Liu Y, Dong S, Wen Z, He W, et al. ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway. *Mol Cancer*. (2017) 16:79. doi: 10.1186/s12943-017-0648-1
82. Chen X, Song M, Zhang B, Zhang Y. Reactive oxygen species regulate T cell immune response in the tumor microenvironment. *Oxid Med Cell Longev*. (2016) 2016:1580967. doi: 10.1155/2016/1580967
83. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol*. (2009) 182:5693–701. doi: 10.4049/jimmunol.0900092
84. Ohl K, Tenbrock K. Reactive oxygen species as regulators of MDSC-mediated immune suppression. *Front Immunol*. (2018) 9:2499.doi: 10.3389/fimmu.2018.02499
85. Pal S, Nandi M, Dey D, Ahammed S, Banerjee S, Santra A, et al. Myeloid-Derived Suppressor Cells: key players mediating immunosuppression via induction of Regulatory T-cell by TGFβ and IL-10 driven pathways in chronic HBV infection. *J Hepatol*. (2017) 66:S541–2. doi: 10.1016/S0168-8278(17)31490-3
86. Bah I, Kumbhare A, Nguyen L, McCall CE, El Gazzar M. IL-10 induces an immune repressor pathway in sepsis by promoting S100A9 nuclear

localization and MDSC development. *Cell Immunol*. (2018) 332:32–8. doi: 10.1016/j.cellimm.2018.07.003
87. O'neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature*. (2013) 493:346–55. doi: 10.1038/nature11862
88. Trikha P, Plews RL, Stiff A, Gautam S, Hsu V, Abood D, et al. Targeting myeloid-derived suppressor cells using a novel adenosine monophosphate-activated protein kinase (AMPK) activator. *Oncoimmunology*. (2016) 5:e1214787. doi: 10.1080/2162402X.2016.1214787
89. Kim SH, Li M, Trousil S, Zhang Y, di Magliano MP, Swanson KD, et al. Phenformin inhibits myeloid-derived suppressor cells and enhances the anti-tumor activity of PD-1 blockade in melanoma. *J Invest Dermatol*. (2017) 137:1740–8. doi: 10.1016/j.jid.2017.03.033
90. Rong Y, Yuan CH, Qu Z, Zhou H, Guan Q, Yang N, et al. Doxorubicin resistant cancer cells activate myeloid-derived suppressor cells by releasing PGE 2. *Sci Rep*. (2016) 6:23824. doi: 10.1038/srep23824
91. Xie Y, Shi X, Sheng K, Han G, Li W, Zhao Q, et al. PI3K/Akt signaling transduction pathway, erythropoiesis and glycolysis in hypoxia. *Mol Med Rep*. (2019) 19:783–91. doi: 10.3892/mmr.2018.9713
92. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*. (2006) 7:606–19. doi: 10.1038/nrg1879
93. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
94. Lindemans C, Coffer P. Regulation of granulocyte apoptosis by phosphatidylinositol 3-kinase. *Biochem Soc Trans*. (2004) 32:480–4. doi: 10.1042/BST0320480
95. Yang KY, Arcaroli J, Kupfner J, Pitts TM, Park JS, Strasshiem D, et al. Involvement of phosphatidylinositol 3-kinase γ in neutrophil apoptosis. *Cell Signal*. (2003) 15:225–33. doi: 10.1016/S0898-6568(02)00063-3
96. Enioutina EY, Bareyan D, Daynes RA. A role for immature myeloid cells in immune senescence. *J Immunol*. (2011) 186:697–707. doi: 10.4049/jimmunol.1002987
97. Lien EC, Dibble CC, Toker A. PI3K signaling in cancer: beyond AKT. *Curr Opin Cell Biol*. (2017) 45:62–71. doi: 10.1016/j.ceb.2017.02.007
98. Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer*. (2006) 6:184–92. doi: 10.1038/nrc1819
99. Damen JE, Ware MD, Kalesnikoff J, Hughes MR, Krystal G. SHIP's C-terminus is essential for its hydrolysis of PIP3 and inhibition of mast cell degranulation. *Blood*. (2001) 97:1343–51. doi: 10.1182/blood.V97.5.1343
100. Ghansah T, Paraiso KH, Highfill S, Desponts C, May S, McIntosh JK, et al. Expansion of myeloid suppressor cells in SHIP-deficient mice represses allogeneic T cell responses. *J Immunol*. (2004) 173:7324–30. doi: 10.4049/jimmunol.173.12.7324
101. Ghansah T. A novel strategy for modulation of MDSC to enhance cancer immunotherapy. *Oncoimmunology*. (2012) 1:984–5. doi: 10.4161/onci.20201
102. Linke M, Fritsch SD, Sukhbaatar N, Hengstschläger M, Weichhart T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett*. (2017) 591:3089–103. doi: 10.1002/1873-3468.12711
103. Zhang C, Wang S, Li J, Zhang W, Zheng L, Yang C, et al. The mTOR signal regulates myeloid-derived suppressor cells differentiation and immunosuppressive function in acute kidney injury. *Cell Death Dis*. (2017) 8:e2695. doi: 10.1038/cddis.2017.86
104. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med*. (2004) 10:594–601. doi: 10.1038/nm1052
105. Porstmann T, Griffiths B, Chung YL, Delpuech O, Griffiths JR, Downward J, et al. PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. *Oncogene*. (2005) 24:6465–81. doi: 10.1038/sj.onc.1208802
106. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell*. (2010) 39:171–83. doi: 10.1016/j.molcel.2010.06.022
107. Chen Y, Qian J, He Q, Zhao H, Toral-Barza L, Shi C, et al. mTOR complex-2 stimulates acetyl-CoA and *de novo* lipogenesis through ATP citrate lyase in HER2/PIK3CA-hyperactive breast cancer. *Oncotarget*. (2016) 7:25224–40. doi: 10.18632/oncotarget.8279
108. Fritsch SD, Weichhart T. Effects of interferons and viruses on metabolism. *Front Immunol*. (2016) 7:630. doi: 10.3389/fimmu.2016.00630
109. Ballou LM, Lin RZ. Rapamycin and mTOR kinase inhibitors. *J Chem Biol*. (2008) 1:27–36. doi: 10.1007/s12154-008-0003-5
110. Weichhart T, Costantino G, Poglitsch M, Rosner M, Zeyda M, Stuhlmeier KM, et al. The TSC-mTOR signaling pathway regulates the innate inflammatory response. *Immunity*. (2008) 29:565–77. doi: 10.1016/j.immuni.2008.08.012
111. Chen W, Ma T, Shen XN, Xia XF, Xu GD, Bai XL, et al. Macrophage-induced tumor angiogenesis is regulated by the TSC2–mTOR pathway. *Cancer Res*. (2012) 72:1363–72. doi: 10.1158/0008-5472
112. Pan T, Zhong L, Wu S, Cao Y, Yang Q, Cai Z, et al. 17β-Oestradiol enhances the expansion and activation of myeloid-derived suppressor cells via signal transducer and activator of transcription (STAT)– 3 signalling in human pregnancy. *Clin Exp Immunol*. (2016) 185:86–97. doi: 10.1111/cei.12790
113. Ma J, Xu H, Wang S. Immunosuppressive role of myeloid-derived suppressor cells and therapeutic targeting in lung cancer. *J Immunol Res*. (2018) 2018:6319649. doi: 10.1155/2018/6319649
114. Nefedova Y, Nagaraj S, Rosenbauer A, Muro-Cacho C, Sebti SM, Gabrilovich DI. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res*. (2005) 65:9525–35. doi: 10.1158/0008-5472.CAN-05-0529
115. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci USA*. (2015) 112:E566–75. doi: 10.1073/pnas.1424927112
116. Sun H, Gong S, Carmody RJ, Hilliard A, Li L, Sun J, et al. TIPE2, a negative regulator of innate and adaptive immunity that maintains immune homeostasis. *Cell*. (2008) 133:415–26. doi: 10.1016/j.cell.2008.03.026
117. Kumar D, Gokhale P, Broustas C, Chakravarty D, Ahmad I, Kasid U. Expression of SCC-S2, an antiapoptotic molecule, correlates with enhanced proliferation and tumorigenicity of MDA-MB 435 cells. *Oncogene*. (2004) 23:612–6. doi: 10.1038/sj.onc.1207123
118. Goldsmith JR, Chen YH. Regulation of inflammation and tumorigenesis by the TIPE family of phospholipid transfer proteins. *Cell Mol Immunol*. (2017) 14:482–7. doi: 10.1038/cmi.2017.127
119. Fayngerts SA, Wu J, Oxley CL, Liu X, Vourekas A, Cathopoulis T, et al. TIPE3 is the transfer protein of lipid second messengers that promote cancer. *Cancer Cell*. (2014) 26:465–78. doi: 10.1016/j.ccr.2014.07.025
120. Fayngerts SA, Wang Z, Zamani A, Sun H, Boggs AE, Porturas TP, et al. Direction of leukocyte polarization and migration by the phosphoinositide-transfer protein TIPE2. *Nat Immunol*. (2017) 18:1353–60. doi: 10.1038/ni.3866.
121. Hart JR, Liao L, Yates JR, Vogt PK. Essential role of Stat3 in PI3K-induced oncogenic transformation. *Proc Natl Acad Sci USA*. (2011) 108:13247–52. doi: 10.1073/pnas.1110486108
122. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. *Nat Rev Cancer*. (2014) 14:736–46. doi: 10.1038/nrc3818
123. Yan DH, Wang JH, Wan XC. TIPE2 is a novel direct target of STAT3 in MDSC and inhibition of its expression on MDSC enhanced T cell activation in tumor. *J Immunol*. (2017) 198:205.
124. Fan Y, Mao R, Yang J. NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. *Protein Cell*. (2013) 4:176–85. doi: 10.1007/s13238-013-2084-3
125. Lian K, Ma C, Hao C, Li Y, Zhang N, Chen YH, et al. TIPE3 protein promotes breast cancer metastasis through activating AKT and NF-κB signaling pathways. *Oncotarget*. (2017) 8:48889–904. doi: 10.18632/oncotarget.16522
126. Lou Y, Zhang G, Geng M, Zhang W, Cui J, Liu S. TIPE2 negatively regulates inflammation by switching arginine metabolism from nitric oxide synthase to arginase. *PLoS ONE*. (2014) 9:e96508. doi: 10.1371/journal.pone.0096508
127. Lou Y, Liu S, Zhang C, Zhang G, Li J, Ni M, et al. Enhanced atherosclerosis in TIPE2-deficient mice is associated with increased macrophage responses to oxidized low-density lipoprotein. *J Immunol*. (2013) 191:4849–57. doi: 10.4049/jimmunol.1300053

128. Trikha P, Carson III WE. Signaling pathways involved in MDSC regulation. *Biochim Biophys Acta*. (2014) 1846:55–65. doi: 10.1016/j.bbcan.2014.04.003
129. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J Exp Med*. (2005) 202:931–9. doi: 10.1084/jem.20050715
130. Zhang Y, Liu Q, Zhang M, Yu Y, Liu X, Cao X. Fas signal promotes lung cancer growth by recruiting myeloid-derived suppressor cells via cancer cell-derived PGE2. *J Immunol*. (2009) 182:3801–8. doi: 10.4049/jimmunol.0801548
131. Donkor MK, Lahue E, Hoke TA, Shafer LR, Coskun U, Solheim JC, et al. Mammary tumor heterogeneity in the expansion of myeloid-derived suppressor cells. *Int Immunopharmacol*. (2009) 9:937–48. doi: 10.1016/j.intimp.2009.03.021
132. Liu B, Qu L, Yan S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. *Cancer Cell Int*. (2015) 15:106. doi: 10.1186/s12935-015-0260-7
133. Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood*. (2011) 118:5498–505. doi: 10.1182/blood-2011-07-365825
134. Harizi H, Grosset C, Gualde N. Prostaglandin E2 modulates dendritic cell function via EP2 and EP4 receptor subtypes. *J Leukoc Biol*. (2003) 73:756–63. doi: 10.1189/jlb.1002483
135. Smyth GP, Stapleton PP, Barden CB, Mestre JR, Freeman TA, Duff MD, et al. Renal cell carcinoma induces prostaglandin E2 and T-helper type 2 cytokine production in peripheral blood mononuclear cells. *Ann Surg Oncol*. (2003) 10:455–62. doi: 10.1245/ASO.2003.06.036
136. Tate DJ, Vonderhaar DJ, Caldas YA, Metoyer T, Patterson JR, Aviles DH, et al. Effect of arginase II on L-arginine depletion and cell growth in murine cell lines of renal cell carcinoma. *J Hematol Oncol*. (2008) 1:14. doi: 10.1186/1756-8722-1-14
137. Sun L, Suo C, Li ST, Zhang H, Gao P. Metabolic reprogramming for cancer cells and their microenvironment: beyond the Warburg Effect. *Biochim Biophys Acta*. (2018) 1870:51–66. doi: 10.1016/j.bbcan.2018.06.005
138. Eruslanov E, Neuberger M, Daurkin I, Perrin GQ, Algood C, Dahm P, et al. Circulating and tumor-infiltrating myeloid cell subsets in patients with bladder cancer. *Int J Cancer*. (2012) 130:1109–19. doi: 10.1002/ijc.26123
139. Kusmartsev S, Eruslanov E, Kübler H, Tseng T, Sakai Y, Su Z, et al. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J Immunol*. (2008) 181:346–53. doi: 10.4049/jimmunol.181.1.346
140. Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P. PGE2-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. *Cancer Res*. (2011) 71:7463–70. doi: 10.1158/0008-5472.CAN-11-2449
141. Jing H, Vassiliou E, Ganea D. Prostaglandin E2 inhibits production of the inflammatory chemokines CCL3 and CCL4 in dendritic cells. *J Leukoc Biol*. (2003) 74:868–79. doi: 10.1189/jlb.0303116
142. O'Sullivan D, Sanin DE, Pearce EJ, Pearce EL. Metabolic interventions in the immune response to cancer. *Nat Rev Immunol*. (2019) 19:324–35. doi: 10.1038/s41577-019-0140-9

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SPARC Is a New Myeloid-Derived Suppressor Cell Marker Licensing Suppressive Activities

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Myeloid-derived suppressor cells (MDSC) are well-known key negative regulators of the immune response during tumor growth, however scattered is the knowledge of their capacity to influence and adapt to the different tumor microenvironments and of the markers that identify those capacities. Here we show that the secreted protein acidic and rich in cysteine (SPARC) identifies in both human and mouse MDSC with immune suppressive capacity and pro-tumoral activities including the induction of epithelial-to-mesenchymal transition (EMT) and angiogenesis. In mice the genetic deletion of SPARC reduced MDSC immune suppression and reverted EMT. *Sparc*$^{-/-}$ MDSC were less suppressive overall and the granulocytic fraction was more prone to extrude neutrophil extracellular traps (NET). Surprisingly, arginase-I and NOS2, whose expression can be controlled by STAT3, were not down-regulated in *Sparc*$^{-/-}$ MDSC, although less suppressive than wild type (WT) counterpart. Flow cytometry analysis showed equal phosphorylation of STAT3 but reduced ROS production that was associated with reduced nuclear translocation of the NF-kB p50 subunit in *Sparc*$^{-/-}$ than WT MDSC. The limited p50 in nuclei reduce the formation of the immunosuppressive p50:p50 homodimers in favor of the p65:p50 inflammatory heterodimers. Supporting this hypothesis, the production of TNF by *Sparc*$^{-/-}$ MDSC was significantly higher than by WT MDSC. Although associated with tumor-induced chronic inflammation, TNF, if produced at high doses, becomes a key factor in mediating tumor rejection. Therefore, it is foreseeable that an unbalance in TNF production could skew MDSC toward an inflammatory, anti-tumor phenotype. Notably, TNF is also required for inflammation-driven NETosis. The high level of TNF in *Sparc*$^{-/-}$ MDSC might explain their increased spontaneous NET formation as that we detected both *in vitro* and *in vivo,* in association with signs of endothelial damage. We propose SPARC as a new potential marker of MDSC, in both human and mouse, with the additional feature of controlling MDSC suppressive activity while preventing an excessive inflammatory state through the control of NF-kB signaling pathway.

Keywords: SPARC, myeloid-derived suppressor cells, breast cancer, neutrophil, neutrophil extracellular traps

INTRODUCTION

Tumor growth implies a systemic state of immune suppression, also characterized by bone marrow (BM) expansion and circulation of myeloid cells able to suppress adaptive immune responses through a variety of mechanisms (1, 2). The so-called myeloid-derived suppressor cells (MDSC) are a heterogeneous pool of myeloid cells, mainly composed by two subsets, the monocytic (M)-MDSC and the polymorphonuclear (PMN)-MDSC, characterized by different phenotypic markers, which are also distinct between human and mouse. The two subsets expand differently and are endowed with different suppressive activities depending on the specific tumor types (3, 4). Persistent tumor release of growth factors and cytokines (such as G-CSF, GM-CSF, and VEGF), promote MDSC production in the BM, whereas tumor release of chemokines (i.e., CCL2, CXCL12) recruits them within the tumor microenvironment (TME) (5, 6). Once in the TME, MDSC acquire suppressive activity through the chronic sensing of inflammatory cytokines and damage-associated molecular patterns (DAMP). PMN-MDSC are phenotypically almost indistinguishable from neutrophils, which also share several functions in favor of tumor growth and dissemination. M-MDSC are instead similar to monocytes and are characterized by high plasticity: in the TME they can differentiate in macrophages and dendritic cells (7, 8).

Neutrophils can chaperone circulating tumor cells that, through a VCAM1-mediated embrace, gain proliferative capacity while in circulation (9). Notably similar interaction, but via beta1 integrin, is retained by PMN even when dying of NETosis, a peculiar type of cell death releasing neutrophils extracellular traps (NETs) (10). NETs are double strand DNA threads decorated with anti-microbial proteins that are extruded by neutrophils to control bacterial and fungi infections. Several stimuli (e.g., IFN, TNF, IL-8, and DAMP) initiate NETosis by binding to neutrophil receptors (e.g., Fc receptors, TLRs) (11). An aberrant NET production has been reported in autoimmune conditions, such as systemic vasculitis and systemic lupus erythematosus (12). Many papers are now describing NET in the context of cancer (10, 13). In solid tumors NET have been shown in clinical samples of triple-negative human breast cancer (TNBC). Using murine models of TNBC Park et al. showed that NET stimulate invasion and migration of breast cancer cells. Inhibiting NET formation or digesting NET with DNAse I *in vivo* reduced lung metastasis. Furthermore, NET can wrap circulating tumor cells (CTC) through a β1 integrin-mediated mechanism or promote cancer cell awakening through extracellular matrix (ECM) remodeling (14, 15). It remains undetermined whether NETs, which are decorated with proteolytic enzymes active on endothelial cells in case of vasculitis, can leave tumor cells unhurt upon their contact.

In this context, we have published that inflammatory neutrophils isolated from subcutaneous agar implants spontaneously extrude NETs (16) and display cytostatic activity against cultured tumor cells (17).

Tumor growth is also associated with aberrant extracellular matrix (ECM) deposition. An increase in collagen content enhances ECM stiffness with consequences on tumor cell survival and migration (18). Other than contributing to the biological and clinical heterogeneity of solid cancers the ECM can directly affect tumor cell as well as immune cell behavior within the TME (19). When activated, immune cells express the ITIM-receptor LAIR-1 that specifically binds to Gly-Pro-Hyp collagen conserved motives (20). The triggering of this receptor activates a negative inhibitory signal that blocks cell activation including NET formation and ROS production (21, 22) We have recently demonstrated that an aberrant ECM deposition characterized by secreted protein acidic and rich in cysteine (SPARC) and high collagen content promotes the recruitment of suppressive myeloid cells (23). SPARC belongs to the class of matricellular proteins with regulatory functions tuning different biological processes, including migration, proliferation, adhesion and cell survival (24). Although not strictly endowed with structural functions, matricellular proteins can control ECM stiffness and composition. In *Sparc*$^{-/-}$ mice, collagen fibers are smaller and disorganized (25, 26) whereas SPARC overexpression promotes collagen fiber deposition and increases ECM stiffness, which in turn activates the process of MDSC recruitment in the TME (23). These data indicate that an ECM rich in SPARC can modulate myeloid cell functions. Less studied and appreciated is the role of matricellular proteins when produced directly by myeloid cells. On this line we have previously shown that another matricellular protein, osteopontin (OPN), when produced by M-MDSC as intracellular protein (iOPN), tunes MDSC suppressive function, modulating the expression of arginase-1, IL-6 and phospho-Stat3 (27). Here we studied the role of SPARC as marker of human and mouse MDSC and its possible regulatory function on their activity.

MATERIALS AND METHODS

Animals, Cell Lines, and *in vivo* Experiments

BALB/cAnNCrl mice (BALB/c) were purchased from Charles River Laboratories (Calco). All experiments involving animals were approved by the Ministry of Health (INT 16_2016, authorization number 288/2017-PR). *Sparc*$^{-/-}$ mice on a BALB/c background were obtained in our laboratory as previously described (25). The mammary carcinoma cell line SN25A was obtained from SPARC-deficient mice that spontaneously developed mammary tumors due to the expression of the rat HER2/neu oncogene (BALB/c; SPARC < tm1Hwe > Tg(MMTV-Erbb2)NK1Mul/J), whereas the N3D cell line was derived from transgenic Her2/Neu mice (BALB/c-Tg(MMTV-Erbb2)NK1Mul/J). Both cell lines were infected with the retroviral vector LXSPARCSH to over-express SPARC and the co-isogenic cell lines, SN25ASP and N3DSP, were obtained (23). Mice were injected into the mammary fat pad with SN25A, N3D, N3DSP (all at the dose of 2x10*5 cells) and SN25ASP (10*6 cells) cell lines and tumors collected when they reached a 10 mm diameter.

Patient Samples and Gene Expression Data

Peripheral Blood was obtained from consecutive breast cancer patients (12 cases) to be surgically resected at Fondazione IRCCS Istituto Nazionale Tumori. The study was approved

by the Medical Ethics Committee (Auth. Number 167/17), and all clinical data were obtained after receiving informed consent, according to institutional rules. Confocal microscopy analysis was performed onto consecutive primary breast tumors surgically resected at Fondazione IRCCS Istituto Nazionale dei Tumori.

Flow Cytometry Analysis

For FACS analysis primary tumors or spleens were collected and maintained in DMEM−10%FBS, then minced and filtered through a 40 μm-pores cell strainer (BD). Red blood cells were removed using ACK lysis buffer (ammonium chloride potassium). Cells were Fc-blocked using CD16/32 antibody (eBioscience) before staining. Antibodies used were: CD45.2; Gr-1; CD11b; Ly6G, and Ly6C (all from eBioscience). Samples were acquired using a BD LSR II Fortessa instrument and analyzed with FlowJo software (TreeStar). All samples are analyzed in single; in each experiment at least 3–4 samples were analyzed for each group.

PBMC Flow Cytometry and Cell Sorting

Blood samples were collected in heparin and peripheral blood mononuclear cells (PBMCs) were obtained by diluting whole blood samples patients 1:2 with PBS 1X and subsequently subjected to a density gradient stratification. Briefly, diluted whole blood samples was carefully layered onto Histopaque-1077 Ficoll (Sigma- Aldrich) and centrifuged at 1,800 rpm for 30 min at room temperature without brake. Finally, the lymphocyte-enriched ring at the interface was transferred into a new collection tube and washed with PBS 1X by centrifugation at 1,200 rpm for 5 min. PBMCs were then stained and analyzed by BD LSR II Fortessa instrument. For MDSC characterization 10^6 PBMC were stained with the following Ab: Lin1 (FITC); HLA-DR (APC eFl780), CD11b (BB700), CD14 (FITC), CD15 (BV650), CD16 (Pe-Cy7), and CD33 (PE) (**Supplementary Table 1**). Total MDSC were sorted from PBMC according to HLA-DR, CD33 and CD11b expression. Cells were sorted using a FACSAria BD Instrument.

MDSC Isolation From Spleen and *in vivo* Tumors for RT-PCR

For MDSC isolation, spleens and mammary lesions from tumor-bearing mice (or naive mice as controls for spleen MDSC), were, minced and filtered to obtain a single cell suspension. Red blood cells were lysed by ACK lysis buffer and MDSC were sorted with FACSAria BD Instrument with the following antibodies: CD45, CD11b, Ly6G, and Ly6C (all from Ebioscience).

RNA Extraction and RT-PCR

For quantitative RT-PCR, myeloid cells were lysed with TRIzol (Invitrogen Life Technologies) and RNA was extracted using the RNeasy Kit (Qiagen). DNA contaminants were removed by treatment with DNase I. cDNA was reverse transcripted from 1 μg of total RNA. PCR was performed using TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and a target gene assay mix containing sequence-specific primers for the *Arginase1, Sparc, TGFb, TNF, NOS-2,* and *Stat3* genes. Gene-specific primers were purchased from Applied Biosystems (Tnf Mm00443260_g1; Arg Mm00475989; Stat3 Mm01219775_m1; Sparc Mm00486332_m1; Vegf Mm01281449; Nos2 Mm00440502_m1; Gapdh Mm99999915_g1). The reactions were set up according to the standard TaqMan qPCR conditions reported in the Applied Biosytems protocol and were performed in duplicate for each sample. The qPCR assays were run using the ABI PRISM® 7900 Fast Real Time PCR system and the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems), and the result data were analyzed with SDS Software 2.3 (Applied Biosystems). The mRNA level of the target genes was quantified by measuring the CT value to determine its relative expression. The results are reported using the fold change in the gene expression of the target genes relative to the internal control gene (GAPDH). The mean-fold change in target gene expression was calculated as 2-ΔΔCT, where DDCT=[(CT,Target - CT,GAPDH)sample-(CT,Target - CT,GAPDH)] internal control.

Immunohistochemistry and Immunofluorescence

Histological and immunohistochemistry analyses of human and mouse tissues were performed as described previously (23). All antibodies that have been used are listed in **Supplementary Table 2**. For double-marker immunofluorescence stainings in which primary antibodies of the same made were adopted, the tyramide signal amplification system Opal multiplex IHC kit (Lot number 2395285, PerkinElmer Inc.) was adopted. Briefly, after deparaffinization, antigen retrieval was performed using microwave heating and a pH9 buffer and the first primary antibody was incubated overnight at 4°C (monoclonal anti-Human Osteonectin/Sparc, Clone ON1-1, 1:500, Life technologies). Immunofluorescence labeling was achieved by incubating with a specific secondary antibody, followed by the addition of one selected Opal fluorophore and microwave treatment in pH9 buffer. The same procedure was repeated for the second primary antibody for 90 min at room temperature (monoclonal anti-Human CD33, Clone PWS44, 1:100, Novocastra), using a different Opal fluoprophore and DAPI nuclear counterstain. All the sections were analyzed under Zeiss Axio Scope A1 optical microscope (Zeiss, Germany) and microphotographs were collected using an Axiocam 503 Color digital camera with the ZEN2 imaging software (Zeiss Germany).

In vitro Suppressive Assay

Myeloid derived suppressor cells were purified using CD11b-conjugated microbeads (for overall population) and Myeloid-Derived Suppressor Cell Isolation Kit [for separation of the two subsets (Miltenyi Biotec)] following the manufacturer's instructions.

For *in vitro* suppression assay, 4 × 105 naïve BALB/c splenocytes have been labeled with CFSE (Carboxyfluorescein Succinimidyl ester; SIGMA Aldrich) and co-cultured with the different MDSC population at different ratio in presence of 2 μg/ml of soluble anti-CD3 and 1 μg/ml of anti-CD28 to activate lymphocytes. Each sample was seeded in triplicate. Proliferation

of CD4 and CD8 T cells has been assessed 2 and 3 days later, by flow cytometry evaluating CFSE dilution in the CD4+ and CD8+ gated populations. Results are shown as percentage of proliferated cells.

ROS Detection

The detection of ROS was performed on the overall population of myeloid derived suppressor cells purified using CD11b-conjugated microbeads using the CellROX® Green Reagent (Life technologies)a fluorogenic probe for measuring ROS in live cells. Oxidation of the cell-permeant dye by ROS generate a bright green fluorescence detectable at FACS.

Evaluation of p50 and p65 Nuclear Translocation

To assess p50 and p65 nuclear translocation BM-derived MDSC were seeded onto poly-D-lysine coated glasses for 2 h in presence of TM supernatants or LPS (10 ng/ml).

Cell permeabilization was obtained after 1 h incubation with PBS 0.1% Triton-X100 (Sigma-Aldrich) plus 5% normal goat serum (Dako Cytomation, Carpinteria, CA USA) and 2% BSA, (Amersham Biosciences, Piscataway Township, NJ USA). Cells were then incubated with rabbit anti-mouse p50 NF-kB (NLS, sc-114; Santa Cruz) or rabbit anti-mouse p65 NF-kB (c-20, sc-372; Santa Cruz). After 1h of incubation at RT, goat anti rabbit AlexaFluor 488 conjugated (LifeTechnologies) were used as secondary antibodies. Nuclei were counterstained with DAPI (Invitrogen, Molecular Probes). Samples were mounted with FluorPreserve Reagent (Calbiochem San Diego, CA USA) and analyzed with a Leica SP8 I laser scanning confocal microscope using a fine focusing oil immersion lens (40X, N.A. 1.3) at 1 Airy Unit resolution and operating in channel mode with 405 and 633 nm excitations. The mean fluorescence intensity of the nucleus was quantified after a freehand drawing considering the nucleus as regions of interest using Image-pro Premium 9.2.

In vitro PMN Cytostasis Assay

PMN were collected from blocks of 2% agarose and 0.2% gelatin in saline 5 days after subcutaneous implant, as described (17). PMN-mediated cytostasis was evaluated in a spectrophotometric assay in 96-well microplates to be read on a microplate spectophotometer. Briefly, after 72 h culture. cells were fixed with 5% formalin and stained with 1% methylene blue in 0.01 M borate buffer, pH 8.5. After eluting the dye from cells with 0.1 N HCl, absorbance was read at 620 nm. The percentage of growth inhibition was calculated as $[1–(A\text{-}B\text{-}C)/(D\text{-}C)] \times 100$, where A, B, C, and D are absorbance of cultures of tumor cells and PMN, of PMN alone, of 10*4 target cells after adhesion for 2 h, and of the dye in wells containing tumor cells cultivated for 72 hr. Results are presented as mean (+SD) of three to six replicates.

Statistical Analysis

Statistical analysis of single treatments was performed using the Mann-Whitney *t*-test. The significance of different combined treatments was assessed through one-way ANOVA with Dunn's multiple comparison test. For other analyses related to MDSC frequency or ELISA data, differences between groups were tested for significance using a two-tailed unpaired *t*-test. Values were considered statistically significant at $p < 0.05$. All of the analyses were performed using Prism software Version 5.0d (GraphPad).

RESULTS

Frequency of MDSC in High-Grade Breast Cancer Patients and Their Expression of SPARC

The peripheral blood (PB) of high-grade breast cancer (BC) patients ($n = 12$) was analyzed for the frequency of early-stage MDSC (eMDSC), identified, by flow cytometry as Lin-HLA-DR-CD33+CD11b+ [as described in (3)]. We found that high-grade BC patients have a significantly increased frequency of eMDSC if compared to healthy donors (HD) (**Figure 1A** and **Supplementary Figure 1A** for gating strategy). Using a different flow cytometry panel that includes HLA-DR, CD33, CD11b, CD14, and CD15 (3, 28) it is possible to define the PMN and M-MDSC subsets in BC patients, being PMN-MDSC HLA-DR-, CD33+CD11b+CD15+ and M-MDSC HLA-DR-CD33+CD11b+CD14+. According to this panel we found that the vast majority of MDSC expanded in BC patients are HLA-DR-CD33+CD11b+ CD14-CD15- therefore not expressing the differentiation markers (**Figure 1A** and **Supplementary Figure 1B** for gating strategy). However, the few M-MDSC were increased in BC patients compared to HD (**Figure 1A**).

To assess the expression of SPARC in circulating human MDSC total HLA-DR-CD33+CD11b+ MDSC were FACS-sorted and evaluated, by real-time (RT)-PCR and confocal microscopy, for the expression of SPARC at RNA and protein level, respectively (**Figures 1B,C**). RT-PCR analysis showed that the expression of SPARC was significantly higher in MDSC obtained from BC patients compared to HD (**Figure 1B**). In line, confocal microscopy analysis confirmed SPARC expression in MDSC of BC patients and less on the fewer HLA-DR-CD33+CD11b+ cells obtained from one HD (**Figure 1C**). To evaluate whether human myeloid cells express SPARC *in situ* in the tumor microenvironment (TME) we performed a double staining confocal microscopy analysis of BC paraffin sections. The representative picture in **Figure 1D** shows SPARC expression in CD33+ cells within the TME.

Next we moved to mouse models to assess whether also murine MDSC express SPARC. To perform this analysis we used 4 different mouse mammary tumors, previously characterized for their different capacity to promote MDSC expansion and activation (23). Indeed, these models were used to demonstrate that SPARC over-expression in BC cells support MDSC development and expansion. In detail, SPARC-deficient (SN25A) or low expressing (N3D) did not promoted MDSC expansion, whereas the SPARC-transduced counterparts (SN25ASP and N3DSP) strongly supported MDSC recruitment and suppressive capacity (23). Using RT-PCR (**Figure 1E**) and immunofluorescence (IF, **Figure 1F** and **Supplementary Figure 1C**, for the M-MDSC subset) analyses we show that SPARC is expressed by both PMN- and

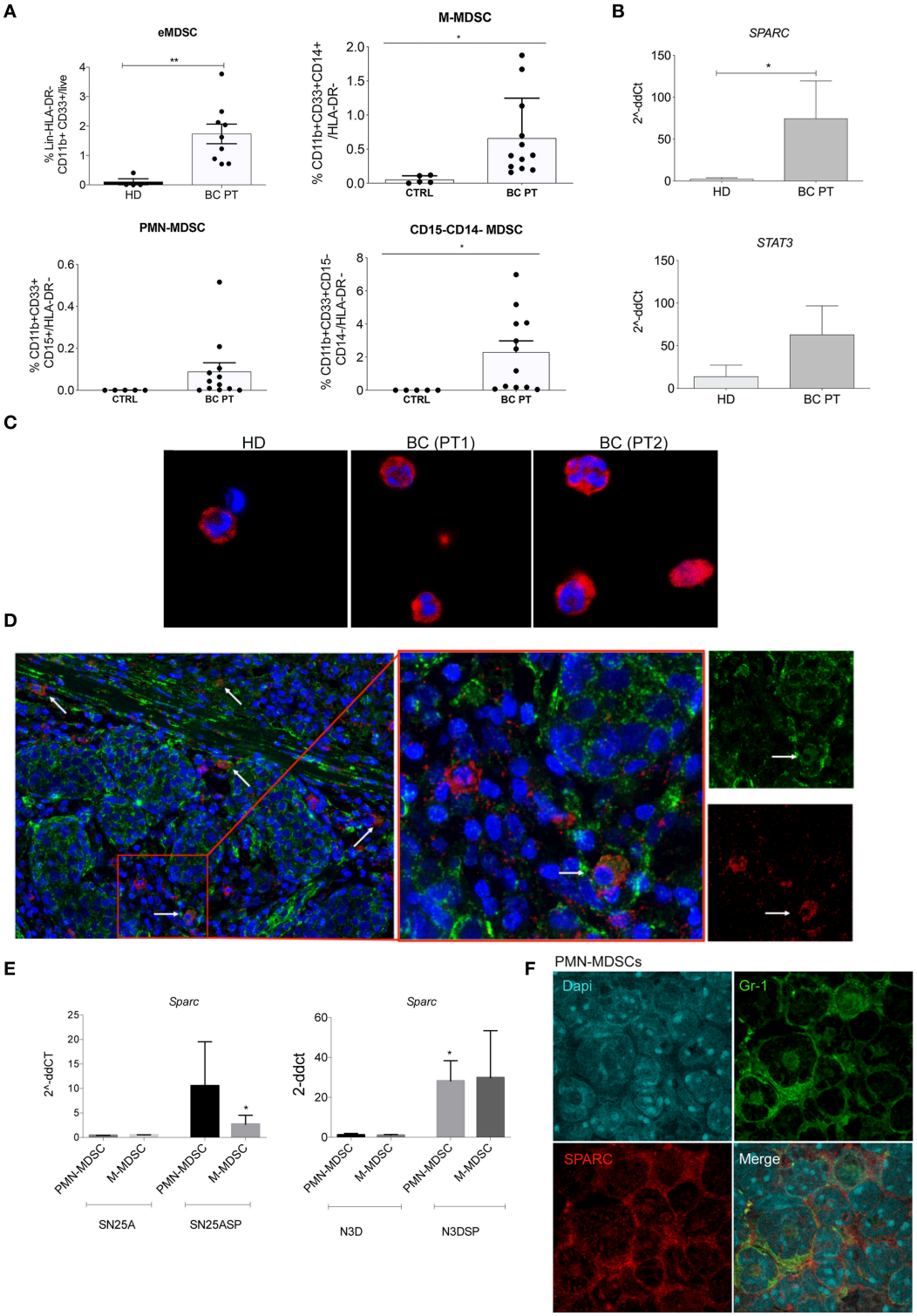

FIGURE 1 | SPARC marks human and murine MDSC. **(A)** Cumulative FACS analysis showing the frequency of early MDSC (eMDSC), PMN- and M-MDSC, and CD11b+CD33+CD14-CD15- in the PB of 12 consecutive BC patients. CD11b+CD33+ eMDSC were defined within HLA-DR-Lin- cell gate. The frequency of e-MDSC was calculated as frequency of CD11b+CD33+ x Frequency of HLA-DR-/100. HLA-DR-CD33+CD11b+CD15+ PMN-MDSC and HLA-DR-CD33+CD11b+CD14+ M-MDSC were identified within the CD11b+CD33+ gate. The CD11b+CD33+ gate was defined on HLA-DR- cells. HLA-DR+ cells were identified within the gate of live cells after doublets exclusion. The frequency of PMN and M-MDSC was calculated as Frequency of CD11b+CD33+ x

(Continued)

FIGURE 1 | Frequency of CD14+ or CD15+/100. The gating strategies are shown in **Supplementary Figure 1D**. **(B)** Semiquantitative real-time PCR analysis for *SPARC* and *STAT3* performed on FACS-sorted MDSC isolated from breast cancer patients (BC PT; $n = 5$) compared to healthy donors (HD; $n = 6$); **(C)** Representative confocal microscopy analysis showing SPARC (red) expression in FACS-sorted HLA-DR-CD33+CD11b+ cells from two representative BC patients and one healthy control. **(D)** Representative confocal microscopy analysis for SPARC (green) and CD33 (red) showing the presence of $CD33^+$ cells expressing SPARC in representative BC patient paraffin sections (white arrows). One representative case is shown. Additional cases are shown in **Supplementary Figure 1**. **(E)** Semiquantitative real-time PCR analysis for *Sparc* performed on murine MDSC subsets sorted from SN25A, SN25ASP, N3D and N3DSP tumors. The Student's *t*-test was used for statistical analysis (*$p < 0.05$; **$p < 0.01$). **(F)** Cytospin preparations of FACS-sorted PMN-MDSC isolated from SN25ASP tumors and stained for Gr1 (green) and SPARC (red). The same staining for M-MDSC is shown in **Supplementary Figure 1C**.

M-MDSC dependently on concomitant tumor expression of SPARC, being associate to N3DSP and SN25ASP, but not to N3D and SN25A tumors. These data point to SPARC as a potential new marker for MDSC. Notably, human BC samples in which SPARC was absent on tumor cells were also devoid of CD33+ cells expressing SPARC, in parallel with mouse results (**Supplementary Figure 1D**).

Myeloid-Derived SPARC Is Required for Epithelial-to-Mesenchymal Transition

To determine the relevance of SPARC when directly produced by MDSC we took advantage from our SN25ASP and N3DSP models in which we showed that the recruitment of MDSC activates an EMT program *in vivo* but not *in vitro* (23).

SPARC-producing SN25ASP cells were injected into SPARC-competent (WT) and SPARC-deficient ($Sparc^{-/-}$) mice. Histopathological analysis showed that tumors developing into $Sparc^{-/-}$ mice had reduced EMT features than those growing into WT recipients (**Figures 2A,B**). Indeed, in WT mice the tumor mass was composed mainly by cells with spindle morphology intermingled with abundant collagenic matrix forming ill-defined nest-like infiltrates. On the contrary, the EMT phenotype was almost entirely reverted in tumors grown into $Sparc^{-/-}$ hosts that showed well-formed nest-like ytumor structures stained for membrane-expressed E-cadherin and reduced frequency of ZEB-1^+ nuclei (**Figures 2A,B**). These data demonstrated that the robust EMT observed in SN25ASP tumors grown in WT mice was likely dependent on microenvironment-derived SPARC. Notably, SN25ASP tumors grew significantly less in $Sparc^{-/-}$ than WT mice (**Figure 2C**), although in presence of reduced EMT. To test whether SPARC produced endogenously by MDSC contributed to EMT, 10^6 MDSC isolated from the spleen of SN25ASP tumor-bearing WT or $Sparc^{-/-}$ mice, were injected, once a week for 4 consecutive weeks (**Figure 2D**), intra-tumorally into SN25ASP lesions grown in $Sparc^{-/-}$ mice. Results show that SN25ASP tumors gained the EMT marker ZEB-1 and lost E-cadherin, thanks to the supplement of SPARC-producing MDSC, despite the SPARC-deficiency in the host (**Figures 2E,F**). In presence of WT MDSC we observed also an increased tumor growth (**Figure 2G**). The data support the hypothesis that SPARC from MDSC is required for immune-mediated EMT.

SPARC Specifies PMN-MDSC Suppressive Functions

To test the functional relevance of SPARC expressed by MDSC, PMN- and M-MDSC subsets were purified from the spleen of tumor-bearing WT or $Sparc^{-/-}$ mice and evaluated for their capacity to inhibit T cell proliferation *in vitro*. PMN-MDSC from $Sparc^{-/-}$ mice had a marked reduced ability to suppress T cell proliferation (**Figure 3A**). Comparison between PMN- and M-MDSC subsets, in this model, was however cumbersome being PMN-MDSC the population that expands mostly in the spleen of tumor-bearing mice, accounting for nearly 80% (77.8 ± 9.6) of the $CD11b^+$ cells, in comparison to the M-MDSC that account for 1% (1.10 ± 0.3). Additionally, the expansion of PMN-MDSC ($Ly6G^{high}$ cells) in $Sparc^{-/-}$ hosts was even higher than in WT counterparts, whereas the M-MDSC (Ly6C ^{high} cells) fraction was reduced (**Figures 3B,C**).

To explain the reduced suppressive capacity of PMN-MDSC from $Sparc^{-/-}$ vs. WT mice, we evaluated the expression of genes that are involved in MDSC suppressive activity in FACS-sorted MDSC from both tumors and spleens (29). Despite their paucity, we also included FACS-sorted M-MDSC obtaining enough material at least for RT-PCR analysis.

Expecting differences, we were surprised of finding similar or higher expression of *Stat3* and *Arginase-I* in $Sparc^{-/-}$ MDSC (both from spleen and tumor) (**Figure 3D** and **Supplementary Figure 2**). Different, however, was *Nos2* that was higher in $Sparc^{-/-}$ than WT PMN-MDSC. NO, the product of NOS2 activity, is a well-recognized pro-inflammatory agent involved, for example, in ulcerative colitis. In support of this idea, *Tnf* mRNA level was higher in $Sparc^{-/-}$ than WT MDSC (**Figure 3D**).

Trying to explain the reduced suppressive activities of $Sparc^{-/-}$ MDSC we evaluate ROS expression in total MDSC (CD11b+ fraction, as described in Melani et al. (6) isolated from the spleen of WT and $Sparc^{-/-}$ tumor-bearing mice. We found a significantly decreased ROS expression by MDSC isolated fro $Sparc^{-/-}$ hosts (**Figures 3E,F**).

To further study the mechanisms behind SPARC induction of a pro-tumoral phenotype in MDSC, we look at NF-kB signaling, as this pathway is involved in monocytes to M-MDSC reprogramming (30–32). To test whether $Sparc^{-/-}$ MDSC have defective NF-kB activation, we evaluated p65 and p50 translocation into the nucleus of BM-differentiated MDSC (28) from WT and $Sparc^{-/-}$ mice, after exposure to tumor supernatants or, as control, to LPS. Confocal microscopy analysis revealed that SPARC-deficient MDSC showed a significantly lower amount of p50 but not of p65 into the nucleus (**Figure 3G** and **Supplementary Figure 3**) than SPARC-competent MDSC, at baseline or when in culture with SN25ASP tumor supernatant (**Figure 3G**). This suggests

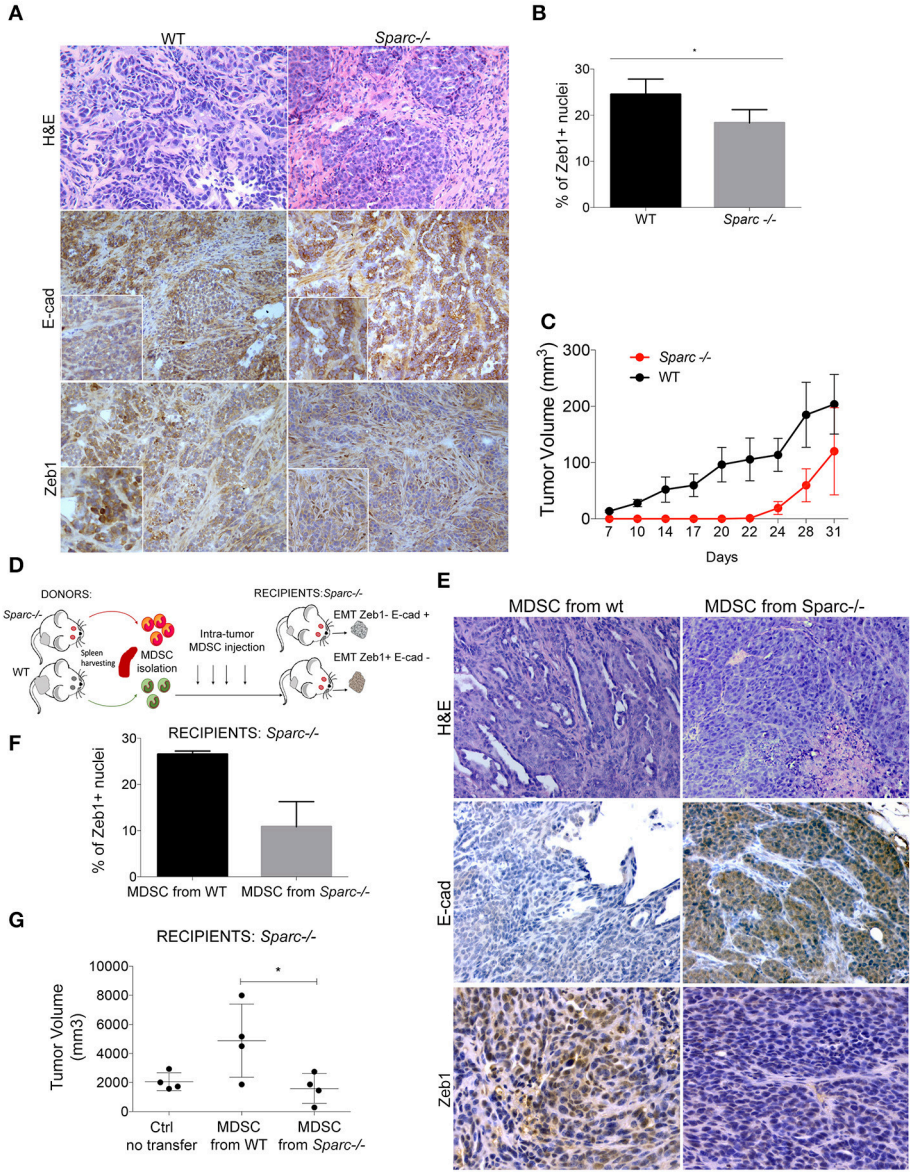

FIGURE 2 | SPARC from MDSC supports EMT. **(A)** H&E and IHC analysis for E-cadherin and Zeb-1 markers performed in SN25ASP tumors obtained from WT and *Sparc*$^{-/-}$ mice. Scale bars, 100 μm. **(B)** Quantitative IHC data for EMT markers are shown as the fraction of positive nuclei for Zeb-1 (*$p < 0.05$; Unpaired *T*-test) in tumors. **(C)** Mean tumor volume of SN25ASP tumors injected in WT and *Sparc*$^{-/-}$ mice. **(D)** Graphical abstract for the MDSC transfer experiment. **(E)** H&E and IHC analysis for E-Cad and ZEB-1 showing the increased expression of EMT markers in SN25ASP tumors grown in *Sparc*$^{-/-}$ mice transferred with WT but not *Sparc*$^{-/-}$ MDSC. **(F)** Quantitative IHC data for EMT markers are shown as the fraction of positive nuclei for Zeb-1. **(G)** Tumor Volume of SN25ASP tumors grown in *Sparc*$^{-/-}$ mice transferred with MDSC from WT and SPARC-deficient mice.

that SPARC-deficient PMN-MDSC may be skewed toward an inflammatory phenotype.

Endogenous SPARC Reverts Tumor-Induced PMN Education Toward Cytostasis

Inflammatory PMN, isolated from subcutaneous agar-implants, exert cytostatic activity toward G-CSF releasing tumor cells (17). Using inflammatory PMN isolated from WT and *Sparc*$^{-/-}$ mice, we evaluated whether tumor cells, according to their ability to promote MDSC differentiation (SN25ASP > SN25A), can inhibit such PMN function and whether PMN from SPARC-competent and -deficient mice are differently susceptible to tumor induced re-education and therefore capable of different cytostatic activity on tumor cells. To this end PMN from WT or *Sparc*$^{-/-}$ mice, were co-cultured with SN25A or SN25ASP tumor cells. Both SN25A and SN25ASP cells lines express G-CSF at similar level, whereas they release different amounts of GM-CSF, IL-6 and COX-2, all significantly higher in the SPARC-over-expressing cell

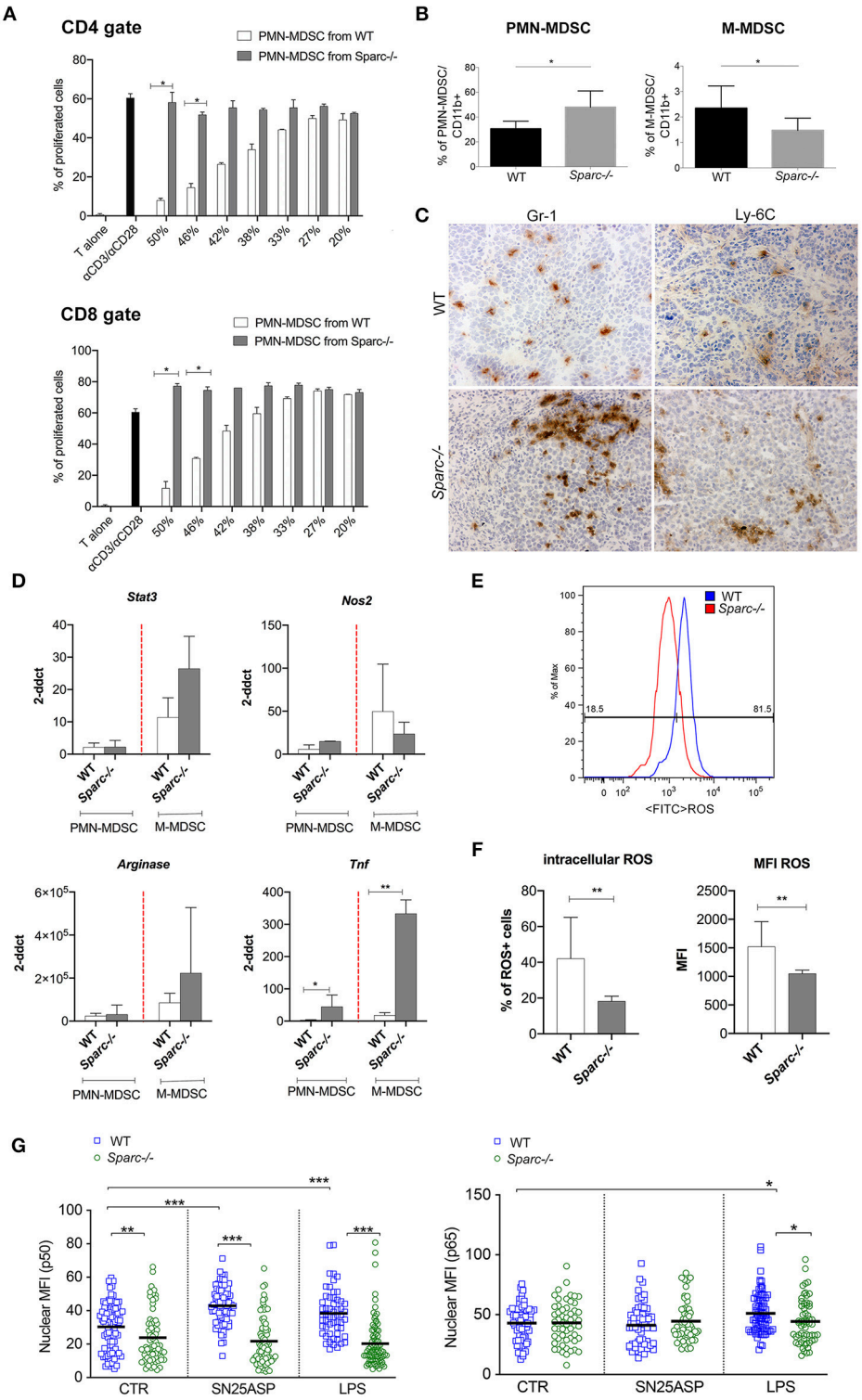

FIGURE 3 | SPARC-deficient MDSC are less suppressive than WT counterparst. **(A)** Immunosuppressive activity of PMN-MDSC isolated from the spleens of WT and *Sparc*$^{-/-}$ tumor-bearing mice evaluated as the ability to suppress a-CD3/a-CD28-induced CD4 and CD8 T cell proliferation *in vitro*. **(B)** FACS analysis of CD11b^{+}, PMN- and M-MDSC performed on peripheral blood of WT and *Sparc*$^{-/-}$ mice injected with the SN25ASP cell line. The Student's t test was used for statistical analysis (*$p < 0.05$). **(C)** IHC analysis of the myeloid markers Gr-1 and Ly-6C performed on WT and *Sparc*$^{-/-}$ tumors, showing the enrichment in Gr-1+ cells in *Sparc*$^{-/-}$ tumors. Scale bars, 100 mm. **(D)** Semiquantitative real-time PCR analysis for *Stat3, Arginase1, Nos2* and *Tnf* genes performed on PMN-MDSC and

(Continued)

FIGURE 3 | M-MDSC subsets sorted from SN25ASP tumors grown in WT and *Sparc*$^{-/-}$ mice ($n = 4$ for per group). The Student's t test was used for statistical analysis (*$p < 0.05$; **$p < 0.01$). **(E)** Representative histogram plots for ROS detection in WT and *Sparc*$^{-/-}$ MDSC. Oxidation of the cell-permeant dye by ROS generate a bright green fluorescence detectable at FACS in the FITC channel. **(F)** Cumulative day showing ROS production by MDSC in terms of percentage of cells oxidating the dye and therefore expressing ROS or the MFI of expression of the oxidated permanent dye. (Student *t*-test **$p < 0.01$). **(G)**. Quantitative data showing p50 and p65 nuclear translocation in MDSC differentiated in presence of G-CSF, GM-CSF, and IL-6 from the BM of WT and *Sparc*$^{-/-}$ mice. MDSC were culture for 2 h in presence of SN25ASP tumor supernatants or LPS (*$p < 0.05$; ***$p < 0.001$).

line (23). Co-culturing these cells with PMN we found that WT PMN had cytostatic activity only against SN25A cells, whereas they were significantly less cytostatic when co-cultured with SN25ASP cells (**Figure 4A**). *Sparc*$^{-/-}$ PMN were similarly able to inhibit the growth of SN25A cells (**Figure 4A**) but contrarily to WT PMN, were also able of cytostasis against SN25ASP cells (**Figure 4A**). These results suggest that SPARC, endogenously produced by MDSC, contributes to tumor-induced education of myeloid cells toward a pro-tumoral phenotype. In line with the pro-inflammatory, anti-tumor activity of myeloid cells from *Sparc*-deficient mice, histological analysis of SN25ASP grown in *Sparc*$^{-/-}$ mice showed features of stromal and vascular damages with vascular lacunae characterized by infiltrating granulocytes undergoing lytic activities (**Figure 4B**).

Altered Tumor Vascularization in SPARC-Deficient Hosts

MDSC can support tumor growth also promoting angiogenesis, for example through the production of VEGF. We investigated whether SPARC can influence tumor angiogenesis as part of their pro-tumorigenic activities. We evaluated VEGF expression by RT-PCR in spleen and tumor MDSC from WT and *Sparc*$^{-/-}$ mice and the serum level of VEGF in SN25ASP tumor-bearing *Sparc*$^{-/-}$ mice, receiving or not a transfer of WT or *Sparc*$^{-/-}$ MDSC. *Vegf* mRNA expression was higher in *Sparc*-deficient PMN-MDSC and M-MDSC (**Figure 5A**), as it was the amount of VEGF in the serum of *Sparc*$^{-/-}$ mice injected with *Sparc*-deficient rather than WT MDSC (**Figure 5B**), suggesting that the lack of SPARC in MDSC may favor tumor vascularization. However, IHC analysis of tumor sections showed a reduced staining of CD31, a marker of endothelial cells, in *Sparc*$^{-/-}$ than in WT mice (**Figures 5C,D**). Therefore, despite a potential increase in tumor angiogenesis because of higher VEGF availability, the concomitant pro-inflammatory nature of *Sparc*$^{-/-}$ PMN likely limits the formation of an efficient vascular network. In favor of this interpretation, IHC analysis performed onto tumor sections shows the presence of PMN destroying the vascular wall of CD31^{+} vessels (**Figure 5E**). Overall these results point to less suppressive *Sparc*$^{-/-}$ MDSC, endowed with cytostatic activities, and of the capacity of damaging tumor vasculature, thus explaining the reduced growth of tumor implanted into *Sparc*$^{-/-}$ mice.

The Absence of SPARC Increases Neutrophil Extracellular Trap Extrusion by PMN-MDSC

NETs are extruded by activated PMN after exposure to a variety of factors (i.e., immune complexes, IFNs, TNF and others). Recently it has been shown that NETs can be extruded by MDSC within the TME via IL-8 stimulation (33). This finding prompted us to assess whether the absence of SPARC could impact NET formation by MDSC expanded in presence of a tumor. To this end PMN-MDSC were isolated from the spleen of tumor-bearing *Sparc*$^{-/-}$ and WT mice, seeded onto poly-D-Lysine coated glasses and stimulated or not with PMA to induce NETosis. We observed that MDSC from tumor-bearing WT and *Sparc*$^{-/-}$ mice were equally able to extrude NETs in presence of PMA, but in its absence only MDSC isolated from *Sparc*$^{-/-}$ mice were able to extrude NETs (**Figure 6**). This might suggest that the sensing of different specific factors or the lack of some brakes produced *in vivo* in the absence of SPARC could differently prime PMN-MDSC for NET formation.

DISCUSSION

Myeloid cells expand within the bone marrow and migrate into the periphery where they are skewed toward MDSC.

We propose that SPARC expression in MDSC is required for their pro-tumor "education." In the absence of endogenous SPARC, MDSC are indeed less suppressive and have reduced capacity to sustain EMT and tumor outgrowth. The reduced suppressive capacity was particularly evident on PMN-MDSC. However, due to the very low amount of M-MDSC in our mammary tumor models we were unable of testing whether SPARC could also influence the activity of the monocytic subset. Although not subverting numerically the PMN-MDSC, in the majority of mouse tumor models a prevalent function is given to M-MDSC (34). However, in few cases the relevance of the suppressive activity of PMN-MDSC has been clearly shown (4). Furthermore, other matricellular proteins have been demonstrated relevant for immunosuppression, such as osteoactivin, also known as glycoprotein nonmetastatic B (GPNMB) (35) and intracellular ostepontin (iOPN) (27).

Different transcription factors have been involved in the acquisition of MDSC suppressive phenotype, among which the best-characterized are STAT3, STAT1 and NF-kB. STAT3 works preferentially on PMN-MDSC and is largely involved in MDSC expansion. In our models, Arginase-I and NOS2, whose expression can be controlled by STAT3, were surprisingly not down-regulated in *Sparc*$^{-/-}$ MDSC, which are low suppressive. Flow cytometry analysis showed equal phosphorylation of STAT3 in WT and *Sparc*$^{-/-}$ MDSC. Also, STAT1, which contributes to suppression, was equally phosphorylated in MDSC from the two strains. Although in contrast with the expected immune suppression, the high STAT3 pathway found in *Sparc*$^{-/-}$ MDSC is in line with the increased VEGF that is regulated by, but

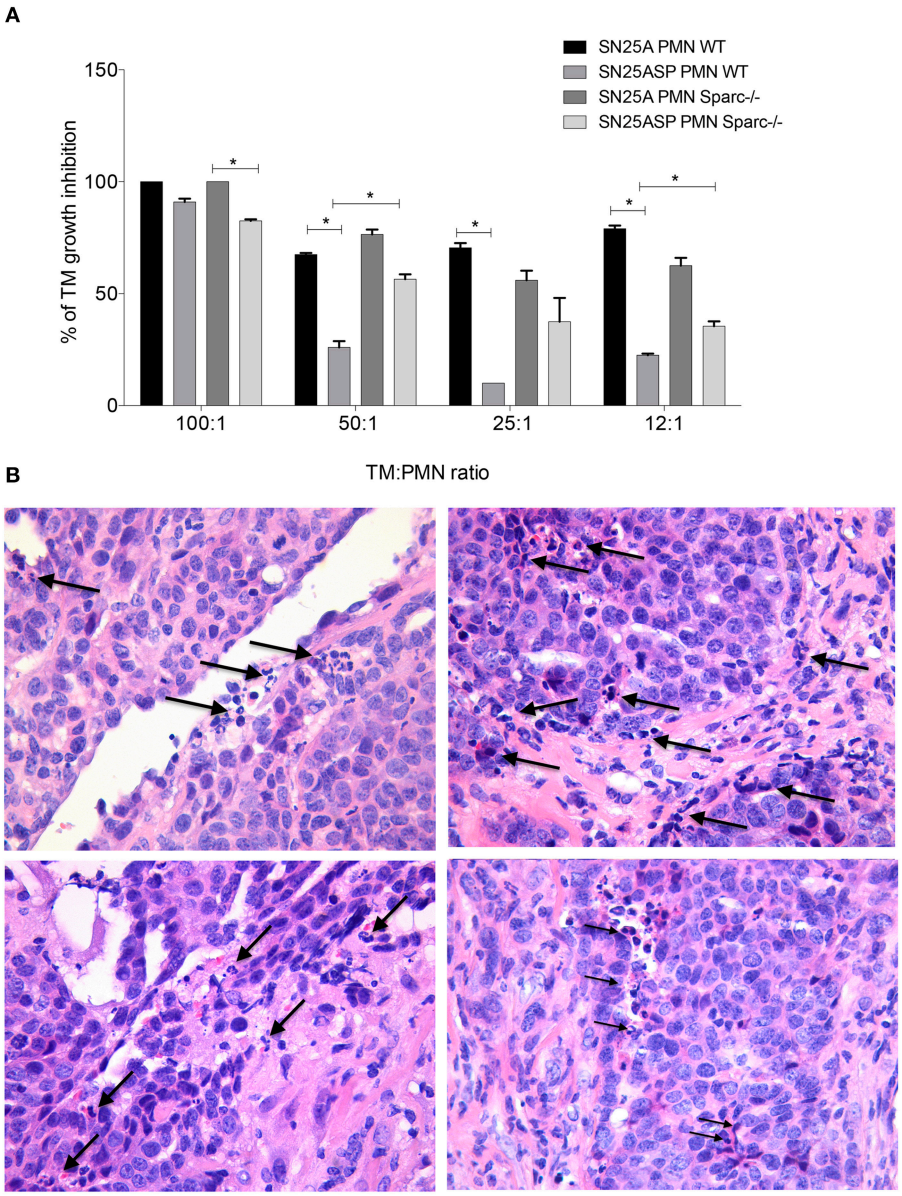

FIGURE 4 | In the absence of SPARC PMN display increased cytostatic activity over tumor cells. **(A)** PMN-mediated cytostatic activity against SN25A and SN25ASP cells. Bars represent the PMN-mediated growth inhibition, means +/- SD of triplicate are shown (*$p < 0.05$). **(B)** H&E analysis showing PMN infiltrating granulocytes undertaking lytic activity (arrows) on both tumor cells and vascular structures.

also regulate, STAT3 (36, 37). We have already described that VEGF expands MDSC suggesting that STAT3 may concur to both suppression and expansion of MDSC, but through uncoupled mechanisms (6). Thus, in *Sparc*$^{-/-}$ mice MDSC expansion can largely depend on VEGF, whereas other mechanisms can account for the reduced MDSC suppression, in presence of key mediators like *Stat3*, *Nos2*, and *Arginase1*. Searching for possible relevant differences between *Sparc*$^{-/-}$ and WT MDSC we found reduced nuclear translocation of the NF-kB p50 subunit, in the former. This may suggest that reduced level of p50 subunits may limit the formation of immunosuppressive p50:p50 homodimers in favor of the p65:p50 inflammatory heterodimers. Supporting this hypothesis, the production of TNF by *Sparc*$^{-/-}$ MDSC was significantly higher than by WT MDSC. Furthermore, very recently Veglia *et al.* reported that the deletion of the fatty acid transport protein 2 (FATP2) abrogated the suppressive activity of PMN-MDSC leaving unaffected the expression of *Arginase 1* and *Nos2* (4). This discrepancy in expression of suppressive genes and MDSC suppressive activity was explained showing reduced PGE2 production by PMN-MDSC from *Fatp2-KO* compared to WT mice. We previously shown that the intracellular retention of SPARC in tumor cells through the over-expression of SCD5, an enzyme that mediated the synthesis of monounsatured fatty acids (MUFA), suppressed tumor growth through an alteration

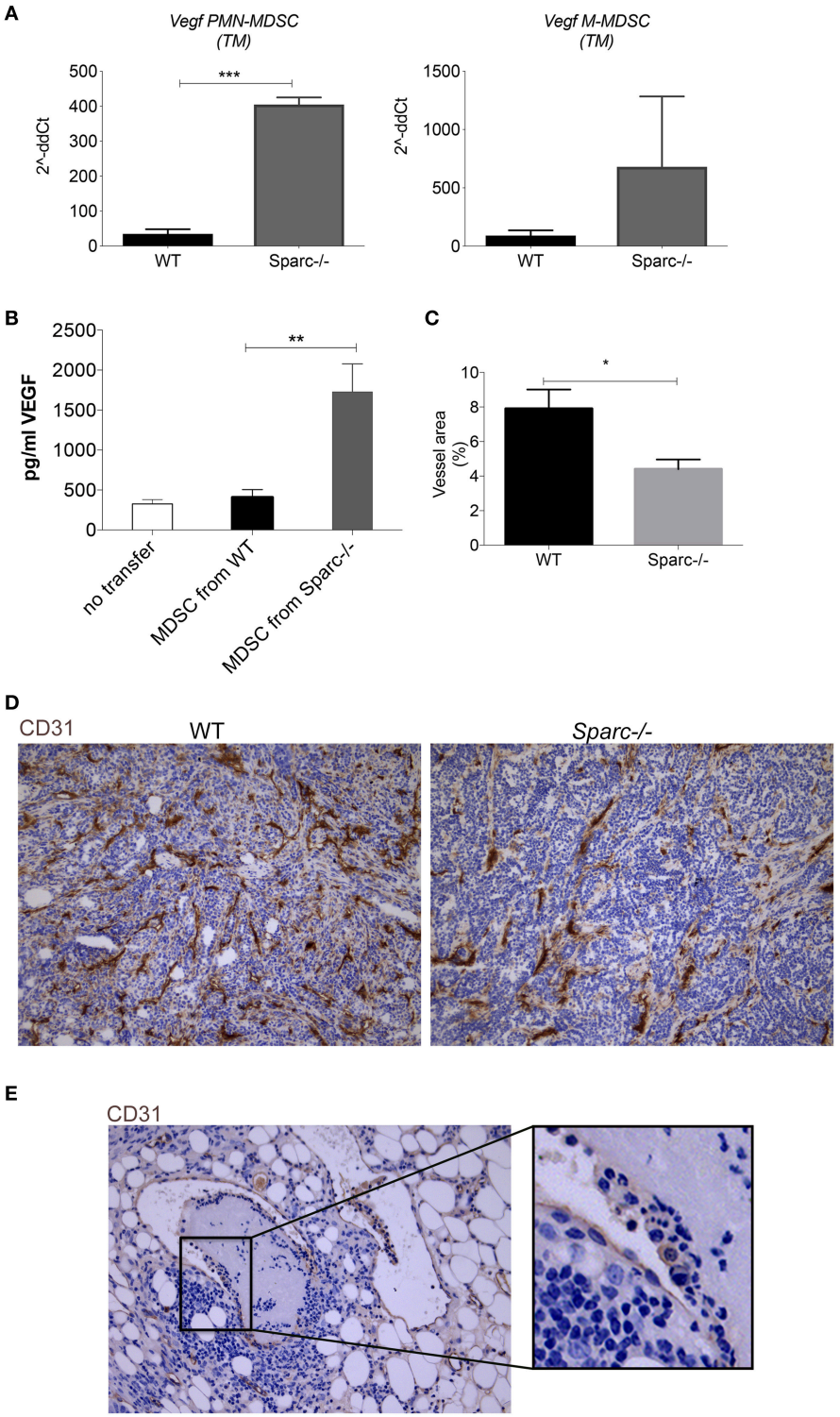

FIGURE 5 | Increased VEGF expression in MDSC from Sparc$^{-/-}$ mice. **(A)** Semiquantitative real-time PCR analysis for *Vegf* performed on PMN-MDSC and M-MDSC subsets sorted from SN25ASP tumors grown in WT and *Sparc*$^{-/-}$ mice ($n = 4$ for per group). The Student's *t*-test was used for statistical analysis (***$p <$ 0.001) **(B)** Representative IHC analysis for CD31 of SN25ASP tumors grown in WT and *Sparc*$^{-/-}$ mice (*$p < 0.05$; **$p < 0.01$) **(C)** Quantification of the vessel areas calculated as (CD31+ area/total tumor area)*100. **(D)** Representative IHC analysis for CD31 in SN25ASP tumor sections from *Sparc*$^{-/-}$ mice. **(E)** The representative picture highlights the presence of PMN (box) destroying the vessel wall.

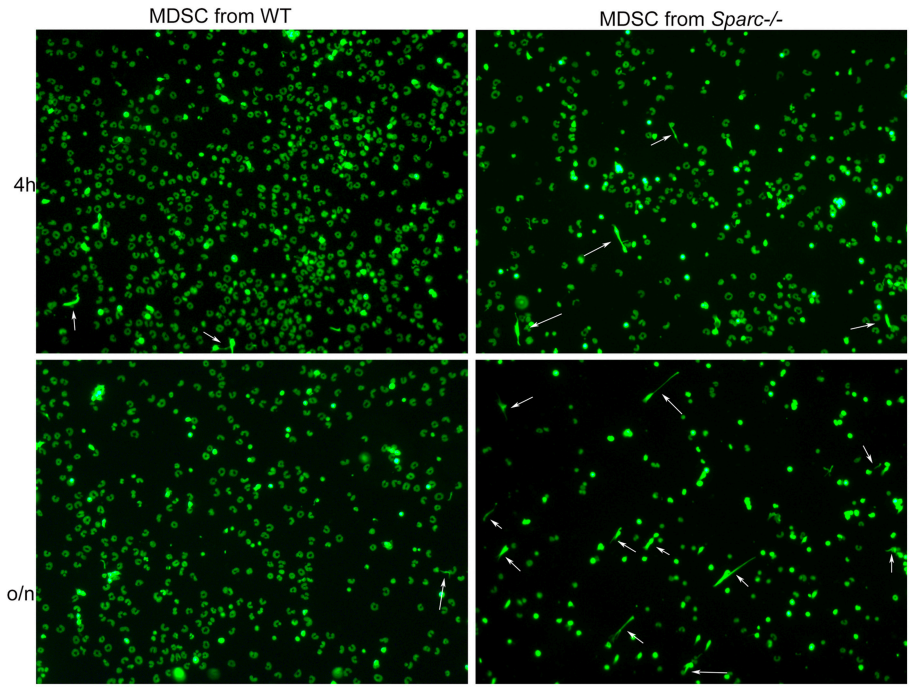

FIGURE 6 | Increased NET formation by Sparc$^{-/-}$ MDSC in comparison to WT counterpart. Representative IF analysis showing increased presence of NETs (white arrows) in *Sparc*$^{-/-}$ MDSC seeded onto poly-D-Lysine coated glasses in presence of the DNA dye Sytox green.

of satured and monounsatured FA balance. The last impacted on tumor growth and metastasis (38). It is reasonable that knocking-down SPARC in MDSC would result in an alteration of FA balance that might ultimately impact on PMN-MDSC functions, as occurred in the case of *Fatp2-KO* mice. Differently from Veglia et al. (4) we found that PMN-MDSC isolated from the spleen of tumor-bearing WT and *Sparc*$^{-/-}$ mice showed a strongly reduced ROS expression in those from *Sparc*$^{-/-}$ mice. However, our findings are in line with the role of fatty acids in the induction of cytosolic and mitochondrial reactive oxygen species (ROS) (38).

Overall these results suggest that the reduced ROS expression combined to the high production of TNF could account for the anti-tumor activity of *Sparc*$^{-/-}$ myeloid cells.

In fact although associated with tumor-induced chronic inflammation (39), TNF if produced at high doses becomes a key factor in mediating tumor-rejection (40). Therefore, it is foreseeable that an unbalance in TNF production could skew MDSC toward an inflammatory, anti-tumor phenotype. Notably, TNF is also required for inflammation-driven NETosis. Indeed, we previously showed impaired NET formation in TNF-KO mice (16) and high TNF in *Sparc*$^{-/-}$ MDSC might explain their increased spontaneous NET formation obtained *in vitro* by seeding MDSC onto poly-D-lysine coated glasses. *In vivo*, spontaneous NETosis was observed mainly in the case of SN25A, a *Sparc*-null tumor when injected into *Sparc*-deficient mice, and less in the case of SN25ASP tumors. The likely explanation should consider that NET formation is negatively regulated by collagens (via LAIR-1) and that collagen is more abundant in SPARC-transduced tumors. This context, associated with a robust inflammatory environment of *Sparc*$^{-/-}$ mice, exacerbates NET formation and their pathogenicity *in vivo*. As occurring in systemic vasculitis (41), in which NETs promote endothelial damage, we found sign of vascular damages in tumors grown in *Sparc*-deficient mice.

Unexpectedly, despite the influence of MDSC-derived SPARC on EMT markers and immune suppression, the tumor volume of SN25ASP tumors injected in WT and Sparc $^{-/-}$ mice was similar at the end, although the differed kinetics of growth that was initially faster in WT mice. Several years ago we published that neutrophils can control tumor growth and favor the elicitation of anti-tumor immune responses (42). Our data suggest that PMN-MDSC from *Sparc*$^{-/-}$ mice behave as N1-like neutrophils rather than MDSC, a condition that allows them to initially control tumor growth until other immune suppressive mechanisms take over (i.e., CD8 T cells exhaustion). Indeed, tumors injected in *Sparc*$^{-/-}$ mice show higher infiltration by CD8 T cells characterized by the expression of multiple markers of exhaustion (not shown).

These results prompt the hypothesis that NET could come in different flavors, according to the context in which they are generated, to sustain either pro-tumor or anti-tumor immunity. An additional level of complexity is introduced by the ECM, as the amount of collagen influences NET formation, despite the presence of an inflammatory environment suitable for such an event.

In conclusion, this paper proposes SPARC as a new potential marker of MDSC, in both human and mouse, with the additional feature of controlling MDSC suppressive activity with the aim of preventing an excessive anti-tumor inflammatory state.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The study on human samples was approved by the Medical Ethics Committee (Auth. Number 167/17), and all clinical data were obtained after receiving informed consent, according to institutional rules. All experiments involving animals were approved by the Ministry of Health (INT 16_2016, authorization number 288/2017-PR).

AUTHOR CONTRIBUTIONS

SS and MC designed the research. GT, CC, BC, LB, PP, AG, and FC performed the experiments. MD and GR provided human blood samples and analyzed clinical parameters. SS, GT, AS, CT, CC, and SS analyzed the data. SS, CC, and MC wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01369/full#supplementary-material

REFERENCES

1. Nagaraj S, Gabrilovich DI. Tumor escape mechanism governed by myeloid-derived suppressor cells. *Cancer Res*. (2008) 68:2561–3. doi: 10.1158/0008-5472.CAN-07-6229
2. Motz GT, Coukos G. Deciphering and reversing tumor immune suppression. *Immunity*. (2013) 39:61–73. doi: 10.1016/j.immuni.2013.07.005
3. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun*. (2016) 7:12150. doi: 10.1038/ncomms12150
4. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature*. (2019) 569:73–8. doi: 10.1038/s41586-019-1118-2
5. Umansky V, Blattner C, Gebhardt C, Utikal J. The role of myeloid-derived suppressor cells (MDSC) in cancer progression. *Vaccines*. (2016) 4:36. doi: 10.3390/vaccines4040036
6. Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. *Cancer Res*. (2007) 67:11438–46. doi: 10.1158/0008-5472.CAN-07-1882
7. Tcyganov E, Mastio J, Chen E, Gabrilovich DI. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol*. (2018) 51:76–82. doi: 10.1016/j.coi.2018.03.009
8. Ugel S, De Sanctis F, Mandruzzato S, Bronte V. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest*. (2015) 125:3365–76. doi: 10.1172/JCI80006
9. Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkountela S, Landin J, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature*. (2019) 566:553–7. doi: 10.1038/s41586-019-0915-y
10. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, et al. Neutrophil extracellular traps sequester circulating tumor cells via beta1-integrin mediated interactions. *Int J Cancer*. (2017) 140:2321–30. doi: 10.1002/ijc.30635
11. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science*. (2004) 303:1532–5. doi: 10.1126/science.1092385
12. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol*. (2018) 18:134–47. doi: 10.1038/nri.2017.105
13. Olsson AK, Cedervall J. NETosis in cancer - platelet-neutrophil crosstalk promotes tumor-associated pathology. *Front Immunol*. (2016) 7:373. doi: 10.3389/fimmu.2016.00373
14. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci Transl Med*. (2016) 8:361ra138. doi: 10.1126/scitranslmed.aag1711
15. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science*. (2018). 361: eaao4227. doi: 10.1126/science.aao4227
16. Sangaletti S, Tripodo C, Chiodoni C, Guarnotta C, Cappetti B, Casalini P, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood*. (2012) 120:3007–18. doi: 10.1182/blood-2012-03-416156
17. Colombo MP, Lombardi L, Stoppacciaro A, Melani C, Parenza M, Bottazzi B, et al. Granulocyte colony-stimulating factor (G-CSF) gene transduction in murine adenocarcinoma drives neutrophil-mediated tumor inhibition *in vivo*. Neutrophils discriminate between G-CSF-producing and G-CSF-nonproducing tumor cells *J Immunol*. (1992) 149:113–9
18. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol*. (2012) 196:395–406. doi: 10.1083/jcb.201102147
19. Sangaletti S, Chiodoni C, Tripodo C, Colombo MP. Common extracellular matrix regulation of myeloid cell activity in the bone marrow and tumor microenvironments. *Cancer Immunol Immunother*. (2017) 66:1059–67. doi: 10.1007/s00262-017-2014-y
20. Lebbink RJ, de Ruiter T, Adelmeijer J, Brenkman AB, van Helvoort JM, Koch M, et al. Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1. *J Exp Med*. (2006) 203:1419–25. doi: 10.1084/jem.20052554
21. Sangaletti S, Tripodo C, Vitali C, Portararo P, Guarnotta C, Casalini P, et al. Defective stromal remodeling and neutrophil extracellular traps in lymphoid tissues favor the transition from autoimmunity to lymphoma. *Cancer Discov*. (2014) 4:110–29. doi: 10.1158/2159-8290.CD-13-0276
22. Geerdink RJ, Hennus MP, Westerlaken GHA, Abrahams AC, Albers KI, Walk J, et al. LAIR-1 limits neutrophil extracellular trap formation in viral bronchiolitis. *J Allergy Clin Immunol*. (2018) 141:811–4. doi: 10.1016/j.jaci.2017.08.031
23. Sangaletti S, Tripodo C, Santangelo A, Castioni N, Portararo P, Gulino A, et al. Mesenchymal transition of high-grade breast carcinomas depends on

extracellular matrix control of myeloid suppressor cell activity. *Cell Rep*. (2016) 17:233–48. doi: 10.1016/j.celrep.2016.08.075
24. Murphy-Ullrich JE, Sage EH. Revisiting the matricellular concept. *Matrix Biol*. (2014) 37:1–14. doi: 10.1016/j.matbio.2014.07.005
25. Sangaletti S, Stoppacciaro A, Guiducci C, Torrisi MR, Colombo MP. Leukocyte, rather than tumor-produced SPARC, determines stroma and collagen type IV deposition in mammary carcinoma. *J Exp Med*. (2003) 198:1475–85. doi: 10.1084/jem.20030202
26. Sangaletti S, Gioiosa L, Guiducci C, Rotta G, Rescigno M, Stoppacciaro A, et al. Accelerated dendritic-cell migration and T-cell priming in SPARC-deficient mice. *J Cell Sci*. (2005) 118(Pt 16):3685–94. doi: 10.1242/jcs.02474
27. Sangaletti S, Tripodo C, Sandri S, Torselli I, Vitali C, Ratti C, et al. Osteopontin shapes immunosuppression in the metastatic niche. *Cancer Res*. (2014) 74:4706–19. doi: 10.1158/0008-5472.CAN-13-3334
28. Strauss L, Sangaletti S, Consonni FM, Szebeni G, Morlacchi S, Totaro MG, et al. RORC1 regulates tumor-promoting "emergency" granulo-monocytopoiesis. *Cancer Cell*. (2015) 28:253–69. doi: 10.1016/j.ccell.2015.07.006
29. Condamine T, Mastio J, Gabrilovich DI. Transcriptional regulation of myeloid-derived suppressor cells. *J Leukoc Biol*. (2015) 98:913–22. doi: 10.1189/jlb.4RI0515-204R
30. Saccani A, Schioppa T, Porta C, Biswas SK, Nebuloni M, Vago L, et al. p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. *Cancer Res*. (2006) 66:11432–40. doi: 10.1158/0008-5472.CAN-06-1867
31. Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol*. (2009) 30:475–87. doi: 10.1016/j.it.2009.07.009
32. Porta C, Ippolito A, Consonni FM, Carraro L, Celesti G, Correale C, et al. Protumor steering of cancer inflammation by p50 NF-kappaB enhances colorectal cancer progression. *Cancer Immunol Res*. (2018) 6:578–93. doi: 10.1158/2326-6066.CIR-17-0036
33. Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). *Clin Cancer Res*. (2016) 22:3924–36. doi: 10.1158/1078-0432.CCR-15-2463
34. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol*. (2008) 181:5791–802. doi: 10.4049/jimmunol.181.8.5791
35. Colombo MP. Is GPNMB the Achilles' Heel of Mo-MDSC While Marking Their Suppressive Activity? *Clin Cancer Res*. (2019) 25:453–4. doi: 10.1158/1078-0432.CCR-18-2334
36. Bartoli M, Platt D, Lemtalsi T, Gu X, Brooks SE, Marrero MB, et al. VEGF differentially activates STAT3 in microvascular endothelial cells. *FASEB J*. (2003) 17:1562–4. doi: 10.1096/fj.02-1084fje
37. Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene*. (2002) 21:2000–8. doi: 10.1038/sj.onc.1205260
38. Bellenghi M, Puglisi R, Pedini F, De Feo A, Felicetti F, Bottero L, et al. SCD5-induced oleic acid production reduces melanoma malignancy by intracellular retention of SPARC and cathepsin B. *J Pathol*. (2015) 236:315–25. doi: 10.1002/path.4535
39. Sangaletti S, Tripodo C, Ratti C, Piconese S, Porcasi R, Salcedo R, et al. Oncogene-driven intrinsic inflammation induces leukocyte production of tumor necrosis factor that critically contributes to mammary carcinogenesis. *Cancer Res*. (2010) 70:7764–75. doi: 10.1158/0008-5472.CAN-10-0471
40. Guiducci C, Di Carlo E, Parenza M, Hitt M, Giovarelli M, Musiani P, et al. Intralesional injection of adenovirus encoding CC chemokine ligand 16 inhibits mammary tumor growth and prevents metastatic-induced death after surgical removal of the treated primary tumor. *J Immunol*. (2004) 172:4026–36. doi: 10.4049/jimmunol.172.7.4026
41. Schreiber A, Rousselle A, Becker JU, von Massenhausen A, Linkermann A, Kettritz R. Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis. *Proc Natl Acad Sci USA*. (2017) 114:E9618–E9625. doi: 10.1073/pnas.1708247114
42. Stoppacciaro A, Melani C, Parenza M, Mastracchio A, Bassi C, Baroni C, et al. Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon gamma. *J Exp Med*. (1993) 178:151–61. doi: 10.1084/jem.178.1.151

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MINI REVIEW
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Long Non-coding RNAs: Regulators of the Activity of Myeloid-Derived Suppressor Cells

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous cell population with potent immunosuppressive functions. They play major roles in cancer and many of the pathologic conditions associated with inflammation. Long non-coding RNAs (lncRNAs) are untranslated functional RNA molecules. The lncRNAs are involved in the control of a wide variety of cellular processes and are dysregulated in different diseases. They can participate in the modulation of immune function and activity of inflammatory cells, including MDSCs. This mini review focuses on the emerging role of lncRNAs in MDSC activity. We summarize how lncRNAs modulate the generation, recruitment, and immunosuppressive functions of MDSCs and the underlying mechanisms.

Keywords: myeloid-derived suppressor cell, long non-coding RNA, inflammation, cancer, immunosuppressive function, accumulation

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INTRODUCTION

The chronic inflammatory conditions typically observed in many diseases can promote the accumulation of myeloid-derived suppressor cells (MDSCs) (1). This heterogeneous cell population with a strong immunosuppressive function has been principally studied in cancer. However, in recent years, the role of MDSCs has been assessed in other conditions, such as diabetes mellitus, obesity, autoimmune diseases, and infectious diseases (2–4).

Different subsets of MDSCs have been reported; in mice, monocyte MDSCs (M-MDSCs) are described as cluster of differentiation (CD)$11b^{+}Ly6G^{-}Ly6C^{+}$ cells, and polymorphonuclear MDSCs (PMN-MDSCs) as $CD11b^{+}Ly6G^{+}Ly6C^{low}$. In humans, M-MDSCs are characterized as $CD11b^{+}CD33^{+}HLADR^{-}CD14^{+}$, whereas PMN-MDSCs are defined as $CD11b^{+}CD33^{+}HLADR^{-}CD15^{+}$ (5). However, other phenotypes have been described in different tumors and infectious diseases (2, 4, 6).

In addition to their high heterogeneity, MDSCs present functional heterogeneity (2). The immunoregulatory functions of MDSCs include the generation of immunosuppressive cells (e.g., regulatory T cells and M2 macrophages) by the production of interleukin (IL)-10; the production of reactive oxygen species (ROS) using the isoforms of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX1, NOX2, NOX3, and NOX4);

and production of reactive nitrogen species, predominantly nitric oxide (NO), by the activation of inducible nitric oxide synthase (iNOS). These reactive species can inhibit the proliferation of T cells, induce the apoptosis of T cells, and reduce both the expression of the ζ chain of T-cell receptors (TCRs), as well as TCR nitration. Moreover, NO can induce the expression of cyclooxygenase 2 (COX2), which regulates the production of prostaglandin-E2, an important molecule that promotes the upregulation of IL-10 and arginase-1 (Arg-1) expression (1). In addition, MDSCs impair the metabolic functions and proliferation of T cells by producing Arg-1, NOS, arginine-glycine amidinotransferase, and L-arginine decarboxylase. The MDSCs can express regulatory molecules, such as programmed death-ligand-1 and Fas ligand to induce the anergy and apoptosis of T cells (1).

The MDSCs originate from common myeloid progenitors in the bone marrow. In addition, extramedullary myelopoiesis in pathologic conditions can generate MDSCs (7, 8). The chronic inflammatory stimuli generated in cancer or infections can induce "emergency myelopoiesis," which is characterized by the expansion of immature myeloid cells to counterbalance the loss of cells (9, 10). Previously, a "two-signal" model of MDSC accumulation was suggested (11), in which the expansion of immature myeloid cells would be supported by growth factors, such as granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and macrophage colony-stimulating factor. However, a "first signal" would also be required to maintain these cells in an undifferentiated state. A "second signal" would promote the activation of immunosuppressive functions, and thereby generate MDSCs (11).

Transcription factors, such as signal transducer and activator of transcription (STAT)3, interferon regulatory factor-8, retinoblastoma protein (RB)1, and CCAAT/enhancer binding protein (C/EBP)β have been linked to the first signal. Stimulation of the immunosuppressive program has been related, for example, to the activation of the transcription factor, nuclear factor-kappa B (NF-kB) through myeloid differentiation factor (MyD)88, the activation of the STAT1 and STAT6 pathways, and endoplasmic reticulum stress-related pathways associated with the transcription factor, C/EBP homologous protein (CHOP) (11, 12). In addition, the factors associated with the two signals could overlap (11, 12). For example, C/EBPβ, a member of the C/EBP leucine zipper domain-containing family, has three isoforms because of different initiation codons: the liver-inhibitory protein (LIP), liver-activating protein (LAP), and full-length liver-activating protein (LAP*). The LAP and LAP* have been considered transcription activators, whereas LIP is considered a repressor or a dominant negative inhibitor of other C/EBP family members (13, 14). Furthermore, C/EBPβ is involved in the regulation of "emergency granulopoiesis" generated by infections or cytokines (15). McPeak et al. showed that reduced expression of C/EBPβ in the myeloid cells of conditional knockout mice was associated with reduced accumulation of MDSCs in a polymicrobial sepsis model (16). In addition, when all CEBPβ isoforms were deleted in hematopoietic lineage cells using tumor mouse models, MDSC accumulation was diminished. Moreover, the MDSCs of these mice showed reduced activity and production of Arg-1 and iNOS proteins (14). *In vitro* studies have suggested that the LAP isoform can bind specific sequences in the regulatory regions of *Arg-1*, *COX2*, *NOX2*, and *iNOS*, and promote their expression in MDSCs. In addition, LIP can supposedly interact with LAP to inhibit its transcription function (17, 18). Thus, C/EBPβ could regulate the expansion and suppression of MDSCs.

The MDSCs have been considered to be central regulators in tumor microenvironments. Elimination of MDSCs by targeting the pathways or molecules involved in their generation, expansion, activation, or recruitment at distant sites, and immunosuppressive function in the local microenvironment could improve the response to cancer treatment (19). Thus, a deeper understanding of the mechanisms involved in the control of these processes is important.

In this context, the epigenetic regulation of the biologic behavior of MDSCs has emerged as a novel field and promising tool in therapy (20). "Epigenetics" refers to heritable changes without variations in DNA sequences, and the study of chromatin (21). Thus, epigenetic mechanisms analyzed in MDSCs involve DNA methylation, histone modifications, and regulation by non-coding RNAs (2, 20, 22). In this review, we summarize current knowledge about the central role of long non-coding RNAs (lncRNAs), a type of non-coding RNA that modulates the generation, recruitment, and immunosuppressive function of MDSCs (**Table 1**). In addition, the underlying molecular mechanisms will be described in some cases.

lncRNAs

The lncRNAs are transcripts larger than 200 nucleotides without coding capacity (31). It has been predicted that the human genome encodes >28,000 lncRNAs, most of which are uncharacterized (32). The biogenesis of lncRNAs has several similarities with the biogenesis of messenger RNA (mRNA) (33). In most cases, lncRNA production is carried out by RNA polymerase II. Modifications include the elimination of introns and the addition of a poly-A tail at the 3′ end, and many (but not all) lncRNAs have a cap in their structure at the 5′ end (33–35).

Based on their genomic localization, lncRNAs can be classified as "intronic" (encoded in the introns of genes), "intergenic" (encoded in the regions between two genes), "enhancer" (encoded in the regions of enhancer promoters), "bidirectional" (encoded in the vicinity of a gene of the opposite strand), "sense-overlapping" (encoded in the introns and exons of different genes in the sense strand of DNA), and "antisense" (encoded in the antisense strands of DNA) (32).

The lncRNAs are highly heterogeneous and have substantial functional versatility based on their capacity to be adapted to different structures and molecular interactions (36). In the nucleus, lncRNAs can act as regulators of transcription (regulating DNA methylation, joining transcription factors, and modifying chromatin); be involved in RNA processing (by means of splicing and antisense alignment); act as "molecular decoys" for proteins, such as p53; and be precursors of microRNAs

TABLE 1 | LncRNAs and their mechanisms implicated in the accumulation and function of myeloid-derived suppressor cells (MDSCs).

Name	Target/mechanism	Biologic effects on MDSCs	Associated disorders	References
lnc-C/EBPβ	lnc-C/EBPβ binding to the LIP isoform promotes interaction with the LAP isoform and stops its activity	lnc-C/EBPβ impedes the immunosuppressive function of MDSCs	Patients with rectal cancer or colon cancer overexpress lnc-C/EBPβ in MDSCs Inflammatory and tumor environments stimulate expression of lnc-C/EBPβ in MDSCs *in vitro* and *in vivo*	(18)
lnc-CHOP	lnc-CHOP binds to the LIP isoform and CHOP, and contributes to LAP activation lnc-CHOP instigates accumulation of the epigenetic marker H3K4me3, commonly associated with transcription activation, in the promoters of Arg-1, NOX2, iNOS, and COX2	lnc-CHOP promotes the immunosuppressive activity and generation of MDSCs	Inflammatory and tumor environments stimulate expression of lnc-CHOP in MDSCs *in vitro* and *in vivo*	(17)
RNCR3	RNCR3 sponges mir-185-5p to increase CHOP expression	RNCR3 promotes the generation and immunosuppressive capacity of MDSCs	Inflammatory and tumor environments stimulate expression of RNCR3 in MDSCs *in vitro* and *in vivo*	(23)
Olfr29-ps1	Olfr29-ps1 sponges miR-214-3p and promotes MyD88 expression N6-methyladenosine modification in Olfr29-ps1 is necessary to exert these effects	Olfr29-ps1 promotes the accumulation and immunosuppressive activity of MDSCs	Increased expression of Olfr29-ps1 is observed in the MDSCs of patients with colon cancer or rectal cancer Inflammatory and tumor environments stimulate expression of Olfr29-ps1 in MDSCs *in vitro* and *in vivo*	(24)
Pvt1	Not described	Pvt1 promotes the immunosuppressive activity of MDSCs	Hypoxic, inflammatory and tumor environments stimulate expression of Pvt1 in MDSCs *in vitro* and *in vivo*	(25)
MALAT1	Not described	MALAT1 negatively regulates MDSC generation	Low expression of MALAT1 has been reported in the PBMCs of patients with lung cancer showing increased proportions of MDSCs	(26)
HOTAIRM1	HOTAIRM1 increases expression of HOXA1, which reduces Arg-1 levels and ROS production in MDSCs	HOTAIRM1 promotes MDSC generation	Decreased expression of HOTAIRM1/HOXA1 has been observed in the MDSCs of patients with lung cancer Negative association between the expression of HOTAIRM1/HOXA1 and presence of MDSCs, as well as Arg1 levels has been observed in patients with lung cancer Positive association between expression of HOTAIRM1/HOXA1 and proportions of Th1/cytotoxic T cells in patients with lung cancer	(27)
RUNXOR	RUNXOR reduces RUNX1 expression	RUNXOR promotes the generation and suppressive activity of MDSCs	Increased expression of RUNXOR and decreased expression of RUNX1 in the MDSCs of patients with lung cancer Positive association between expression of RUNXOR and presence of MDSCs, as well as Arg-1 levels in patients with lung cancer; in contrast, a negative association has been observed with RUNX1 Negative association between RUNXOR expression and proportions of Th1/cytotoxic T cells in patients with lung cancer	(28)
HOTAIR	Not described	Increased expression of HOTAIR in hepatocellular carcinoma cell lines promotes MDSC generation	Negative association between HOTAIR expression and the presence of MDSCs in the blood of HPV-positive patients with HNSCC	(29, 30)
PROM1 CCAT1 MUC19	Not described	Not determined	Negative association between expression of PROM1, CCAT1, and MUC19, and the presence of MDSCs in the blood of patients with HPV-positive HNSCC	(29)

(miRNAs). In the cytoplasm, they can act as molecular decoys for miRNAs, and "scaffolds" for cytoplasmic proteins (37).

Although only a small proportion of all identified lncRNAs have been studied in depth, they are fundamental in many cellular contexts and diverse biological processes (38). In this context, these functional transcripts regulate the differentiation of megakaryocytes, granulocytes, monocytes, and macrophages, and modulate the inflammatory response (39, 40).

EXPRESSION AND FUNCTION OF LNCRNAS IN MDSCS

lnc-C/EBPβ

The lnc-C/EBPβ (also named E130102H24Rik) is an intergenic lncRNA conserved in mice and humans that is encoded in chromosome 1 and chromosome 4, respectively. In addition, it has been found predominantly in cellular nuclei. High expression of lnc-C/EBPβ in the myeloid cells (e.g., macrophages and dendritic cells) of mice has been reported. Importantly, differential expression of lnc-C/EBPβ has been observed in mouse MDSCs if variations in the inflammatory environment occur. Moreover, IL-6 can promote lnc-C/EBPβ expression in MDSCs. Expression of lnc-C/EBPβ has also been reported in M-MDSCs, as well as the PMN-MDSCs of patients with colon cancer or rectal cancer (18). The lnc-C/EBPβ can inhibit expression of enzymes, such as Arg-1, iNOS, NOX2, and COX2, in mouse MDSCs and human MDSC-like cells, resulting in lower concentrations of their metabolic products.

Overexpression of lnc-C/EBPβ in MDSCs promotes the expression of interferon (IFN)-γ in T cells. Tumors in murine models show slower growth if mice are treated with MDSCs overexpressing lnc-C/EBPβ, and tumor-infiltrating T cells demonstrate increased expression of IFN-γ, as compared with controls (18). Thus, lnc-C/EBPβ can attenuate the immunosuppressive function of MDSCs. The suggested mechanism by which this is achieved is the binding of lnc-C/EBPβ to C/EBPβ (specifically to the LIP isoform), which promotes the interaction of LIP with the transcription activator LAP. These interactions prevent the accumulation of LAP in the promoters of *Arg-1*, *iNOS*, *NOX2*, and *COX2*, resulting in decreased expression of these enzymes (18).

Expression of lnc-C/EBPβ can block the generation of murine M-MDSCs (18). Because lnc-C/EBPβ has conserved expression and negatively regulates the differentiation and immunosuppressive activity of MDSCs, it could be a potential target for future studies in immunotherapy. To our knowledge, only one report has analyzed this lncRNA. Hence, further studies on the additional functions of lnc-C/EBPβ in various physiological processes and cancer, as well as the molecular mechanisms involved in its regulation of the immune response are necessary.

lnc-CHOP

The lnc-CHOP (also named GM16727) is an intronic lncRNA that has not been widely characterized. It is encoded in chromosome 11 and localized in cellular nuclei. Interleukins, such as IL-6 and tumor necrosis factor (TNF)-α, and tumor-associated factors can induce the increased expression of lnc-CHOP in mouse MDSCs. Overexpression of lnc-CHOP promotes the expression of Arg-1, NOX2, iNOS, and COX2 and their metabolites in MDSCs, and contributes to the reduction of IFN-γ produced by T cells (17). Thus, lnc-CHOP fosters the immunosuppressive activity of mouse MDSCs.

Furthermore, lnc-CHOP positively regulates MDSC generation and promotes tumor growth in murine models. It has been suggested that lnc-CHOP binds to the transcription factor CHOP and the C/EBPβ isoform, LIP. This allows the activation of LAP and its accumulation in the promoters of target genes, thereby promoting the expression of Arg-1, NOX2, NOS2, and COX2. In addition, increased expression of these enzymes could be the result of enrichment of trimethylation of the amino acid, lysine, at position 4 in the histone H3 (H3K4me3) marker of their promoter regions. The H3K4me3 marker is usually enriched at active chromatin regions and its accumulation is promoted by overexpression of lnc-CHOP in MDSCs (17). The lnc-CHOP could use different mechanisms to promote the accumulation and activation of MDSCs. The conservation and role of lnc-CHOP in human MDSCs, as well as its contribution in tumor biology, has yet to be determined. Future studies could ascertain the additional functions of lnc-CHOP and its potential applications.

Retinal Non-coding RNA (RNCR)3

The RNCR3 is an intergenic lncRNA that is highly conserved in mammals (in which it is also known as LINC00599) (41). The RNCR3 expression is reportedly related to glioblastoma, prostate cancer, atherosclerosis, and retinal microvascular abnormalities (41–44). Mouse MDSCs express nuclear and cytoplasmic RNCR3, the expression of which is increased in mice with tumors. In addition, IL-6 induces the increased expression of this lncRNA in the MDSCs of mice (23). Downregulation of RNCR3 expression prevents MDSC differentiation *in vitro* and *in vivo*, whereas RNCR3 expression promotes the preferential differentiation of PMN-MDSCs. Importantly, RNCR3 contributes to the immunosuppressive function of MDSCs to induce the expression of Arg-1 and iNOS *in vitro*.

Furthermore, IFN-γ production by T cells is increased in the presence of MDSCs with reduced expression of RNCR3. A tumor model in mice treated with MDSCs down-regulating the expression of RNCR3 showed increased tumoral growth (23). One possible mechanism for this is the use of RNCR3 to "sponge" mir-185-5p. The latter impedes MDSC generation and the production of INOS and Arg-1 by targeting CHOP. In the presence of RNCR3, mir-185-5p binds to it preferentially, resulting in the upregulation of CHOP expression (23). The immunosuppressive function of MDSCs is promoted by CHOP, as CHOP-deficient MDSCs show increased expression of the LIP isoform and reduced binding of C/EBPβ to promoters of Arg-1 and IL-6. This results in the reduced expression of IL-6 and activation of STAT3, as well as impaired immunosuppressive function (45). Thus, RNCR3 supports the accumulation and immunosuppressive program of MDSCs. Additional studies could determine whether RNCR3 exerts biological effects on human MDSCs for potential applications against chronic inflammatory diseases in humans.

Olfactory Receptor 29, Pseudogene 1 (Olfr29-ps1)

The Olfr29-ps1 is a lncRNA in mice (OR1F2P in humans) that has not been characterized previously. It is conserved and expressed in the nuclei and cytoplasm of murine and human MDSCs and macrophages. Tumor-associated factors and IL-6 can increase the expression of Olfr29-ps1 in the MDSCs of mice.

Mononuclear cells with a MDSC phenotype in patients with colon cancer or rectal cancer have shown increased expression of Olfr29-ps1 (24). The overexpression of Olfr29-ps1 in bone marrow cells (BMCs) has been shown to promote the generation of mouse M-MDSCs in a differentiation model using cellular cultures, and an *in vivo* BMC chimera model. In addition, augmented accumulation of M-MDSCs has been observed in the tumors and spleen of a tumor model in mice with Olfr29-ps1-overexpressing MDSCs. The immunosuppressive activity of human and murine MDSCs is increased by Olfr29-ps1 overexpression (24).

In vitro analysis showed that IFN-γ production by T cells is reduced in the presence of Olfr29-ps1-overexpressing MDSCs, and that these MDSCs show increased protein expression of Arg-1, COX2, NOX2, and iNOS, as well as increased production of their metabolites. Tumors in a mouse model with Olfr29-ps1-overexpressing MDSCs show greater growth and fewer infiltrating T (especially CD^{8+}) cells. *In vitro* analyses suggest that the effects generated by Olfr29-ps1 could be explained (at least in part) by its capacity to sponge miR-214-3p (24). The latter inhibits the expression of the mRNA and protein expression of MyD88, so interactions between Olfr29-ps1 and miR-214-3p result in the augmented expression of MyD88 (24). Thus, the immunosuppressive activity of MDSCs is promoted by MyD88.

Interestingly, modification of N6-methyladenosine in regions of the Olfr29-ps1 sequence is essential for the stability and function of Olfr29-ps1 in MDSCs. This modification is common in mRNAs and generated by methyltransferases, such as methyltransferase-like (METTL)3. Notably, the downregulation of METTL3 expression reduces Olfr29-ps1 production, as well as the immunosuppressive activity and generation of MDSCs (24). Mechanistically, these observations are important because modification of N6-methyladenosine is reversible and could have potential therapeutic benefits. However, only additional research will show whether this is possible.

Plasmacytoma Variant Translocation (Pvt)1

The Pvt1 is an intergenic lncRNA. It is conserved in humans and mice. Notably, it is over-expressed in several human cancers, including melanoma, cervical cancer, gastric cancer, prostate cancer, hepatocellular cancer, esophageal cancer, and acute myeloid leukemia (25, 46, 47). Tumor-infiltrating PMN-MDSCs and M-MDSCs show increased expression of Pvt1 in tumor mouse models, and overexpression in the splenic MDSCs of those mice. In addition, the presence of IL-6 increases the expression of Pvt1 in PMN-MDSCs generated in cultures. Interestingly, hypoxic conditions and expression of hypoxia-inducible factor (HIF)-1α increases Pvt1 production in PMN-MDSCs *in vitro* (25). Thus, inflammatory and tumor microenvironments could promote the increased expression of this lncRNA in MDSCs.

Downregulation of Pvt1 expression in PMN-MDSCs can induce reduced production of ROS and Arg-1 activity, as well as a slight increase in T-cell proliferation in co-cultures. A tumor mouse model treated with Pvt1-down-regulating PMN-MDSCs showed reduced growth in the generated tumors and a modest increase in the number of CD8+ T cells producing IFN-γ in lymphatic nodules (25). These results suggest that Pvt1 promotes immunosuppressive activity in PMN-MDSCs. Whether Pvt1 modulates the immunosuppressive functions of human MDSCs warrants future exploration. In addition, the molecular mechanisms involved in MDSC regulation by this lncRNA should be investigated.

Interestingly, similar microenvironmental factors (e.g., IL-6 or tumor-associated factors) can induce the overexpression of lnc-C/EBPβ, lnc-CHOP, Olfr29-ps1, Pvt1, and RNCR3 in MDSCs. These microenvironmental factors produce contrasting effects because lnc-CHOP, Olfr29-ps1, Pvt1, and RNCR3 promote, whereas lnc-C/EBPβ prevents, the immunosuppressive functions and differentiation of MDSCs (17, 18, 23–25). These actions could indicate "fine tuning" of gene regulation and the importance of lncRNAs in the control of the biological behavior of MDSCs. In addition, the final biological effect could result in crosstalk among the diverse pathways regulated by lncRNAs.

Metastasis-Associated Lung Adenocarcinoma Transcript (MALAT)1

The MALAT1 (also named nuclear-enriched abundant transcript-2) is a nuclear intergenic lncRNA. It is highly conserved among species and involved in various diseases. The MALAT1 is considered an oncogene because it can promote the proliferation, invasion, and metastasis of many types of human cancer cells (48). Thus, this lncRNA has been studied to develop new strategies in the diagnosis and treatment of cancer (48). Using an *in vitro* differentiation model, Zhou and colleagues recently reported that the reduced expression of MALAT1 in peripheral blood mononuclear cells (PBMCs) promotes their differentiation to MDSC-like cells. Interestingly, the reduced expression of MALAT1 has been reported in the PBMCs of patients with lung cancer, as well as an increased proportion of MDSCs (26). Thus, MALAT1 could negatively regulate the differentiation of MDSCs in patients with lung cancer. More studies evaluating the role and mechanisms through which MALAT1 regulates MDSC differentiation in different diseases could lead to new directions in potential therapeutics.

HOXA Transcript Antisense RNA Myeloid-Specific (HOTAIRM)1

The HOTAIRM1 is an intergenic lncRNA localized between homeobox (*HOX*)*A1* and *HOXA2* genes, and is expressed preferentially in the myeloid lineage. It has been associated with glioblastoma and myeloid leukemia, as well as colorectal, pancreatic, lung, and breast cancer (49, 50). Importantly, HOTAIRM1 regulates the differentiation of myeloid cells (27, 50). Reduced expression of HOTAIRM1 has been reported in the MDSCs of tumors of patients with lung cancer. In addition, the overexpression of HOTAIRM1 has been shown to reduce the differentiation of MDSCs and production of Arg-1 in cellular cultures using human PBMCs (27). Consistent with those data, diminished expression of HOTAIRM1 was found in the PBMCs of patients with lung cancer, as well as increased proportions of MDSCs. Moreover, a negative association was observed between HOTAIRM1 expression and the presence of MDSCs, as well as Arg-1 production; whereas a positive association was observed

with respect to the percentage of T-helper (Th)1 cells and cytotoxic T cells in the same patients (27).

The mechanism by which HOTAIRM1 regulates MDSCs could be associated with *HOXA1* expression. The HOTAIRM1 can induce HOXA1 expression in MDSCs, which reduces Arg-1 expression and ROS production. In addition, increased expression of HOXA1 has been shown to reduce tumor growth, decrease the percentage of MDSCs, and enhance the immune response in a tumor mouse model. Moreover, a positive association has been observed between the expression of HOTAIRM1 and HOXA1 in patients with lung cancer (27). These observations suggest that HOTAIRM1 inhibits the differentiation and suppressive activity of human and mouse MDSCs. Further studies analyzing the effects of HOTAIRM1 on MDSCs in other tumor types should be conducted. In addition, the mechanisms involved in the reduced expression of HOTAIRM1 in lung cancer have yet to be determined.

Runt-Related Transcription Factor-1 Overlapping RNA (RUNXOR)

The RUNXOR is an intragenic lncRNA that has been very rarely studied. It is localized in the locus of the runt-related transcription factor (*RUNX*)*1* gene, and its expression is increased in the bone marrow of patients with acute myeloid leukemia (51). The MDSCs generated in cell cultures using the PBMCs and MDSCs of tissue from patients with lung cancer express high levels of RUNXOR. Furthermore, the down-regulated expression of this lncRNA in PBMCs disturbs their differentiation to MDSCs in a cell-culture model. Moreover, Arg-1 expression is reduced if RUNXOR expression is decreased in MDSCs. Thus, RUNXOR is involved in promoting the generation and immunosuppressive function of MDSCs (28).

Interestingly, the increased expression of RUNXOR has been reported in the PBMCs of patients with lung cancer, and a positive correlation has been described between RUNXOR expression and the presence of MDSCs, as well as Arg-1 production in such patients. In contrast, a negative correlation has been observed between RUNXOR expression and the percentage of both Th1 cells and cytotoxic T cells (28). It has been suggested that RUNXOR exerts its biologic effects on MDSCs through its target RUNX1. Wang and co-workers suggested that RUNXOR binds the enhancer of zeste homolog 2 (histone H3K27 methyltransferase component of polycomb repressive complex 2) and RUNX1 protein to the RUNX1 promoter; and the RUNXOR promoter could compete with the RUNX1 promoter for the transcription machinery (51). Thus, RUNXOR could reduce RUNX1 expression in MDSCs *in vitro* and in patients with lung cancer. Reduced expression of RUNX1 in mouse MDSCs promotes the production of Arg-1, iNOS, and ROS *in vitro*. Moreover, RUNX1 expression induces the differentiation of MDSCs into myeloid cells with a mature phenotype (52). Therefore, RUNXOR could promote the expansion and immunosuppressive activity of MDSCs in lung cancer.

Evidently, MALAT1, HOTAIRM1, and RUNXOR regulate important biological activities (e.g., expansion, differentiation, and immunosuppressive functions) of MDSCs in lung cancer. Hence, these lncRNAs could offer opportunities for potential therapeutic applications against lung cancer; nevertheless, a considerable amount of research would be necessary.

Hox Antisense Intergenic RNA (HOTAIR)

The HOTAIR is an oncogenic lncRNA positively associated with initiation, growth, angiogenesis, progression, drug resistance, and poor prognosis in cancer (53). The expression of HOTAIR has been indirectly related to MDSC recruitment. A negative association has been reported between HOTAIR expression and the proportion of MDSCs in the blood samples of patients with human papillomavirus-positive head and neck squamous cell carcinoma (HPV-positive HNSCC) (29), but a causal relationship has not been established. Furthermore, HOTAIR overexpression in cell lines of hepatocellular carcinoma can induce increased production of the C-C motif chemokine ligand (CCL)2 (30). The CCL2 is not a specific chemokine for MDSCs and is a chemoattractant for several tumor-related myeloid cells (including monocytes and tumor-associated macrophages). Hence, HOTAIR expression could exert a more generalized function by promoting inflammation and immunosuppression within the tumor microenvironment. In addition, using a differentiation model of PBMCs from human donors, cell lines of hepatocellular carcinoma overexpressing HOTAIR promoted MDSC differentiation in co-cultures (30). Thus, HOTAIR expressed by tumor cells could positively regulate MDSC generation *in vitro*; nevertheless, the associated molecular mechanisms have not been determined. Studies are needed to ascertain the functional role of HOTAIR in the recruitment or differentiation of MDSCs.

Other lncRNAs

In addition to HOTAIR, three other lncRNAs have been negatively associated with MDSCs in the blood samples of patients with HPV-positive HNSCC: prominin (PROM)1, colon cancer associated transcript (CCAT), and mucin (MUC)19 (29). However, the molecular mechanisms implicated in the recruitment or expansion of MDSCs have not been determined, because a direct molecular role of these lncRNAs in MDSC biology has not been reported. In future studies, these lncRNAs could be evaluated as potential biomarkers in patients with HPV-positive HNSCC, because the proportion of MDSCs is increased in these patients as compared with precancerous lesions and normal oral mucous tissues (29).

To our knowledge, only one study has focused on the relationship between lncRNAs and MDSCs in non-cancer-related diseases. The lncRNA expression was analyzed in the MDSCs generated in mice infected with *Echinococcus granulosus* (54) (the causal agent of cystic echinococcosis in humans). This zoonotic disease principally affects the liver and lungs. In mice infected with *E. granulosus*, the expansion of MDSCs that down-regulate the activity of T cells has been reported (55). These MDSCs, in the presence of this infectious agent, showed 649 differentially expressed lncRNAs. Bioinformatics analyses based on mRNA expression revealed alterations in biologic processes (e.g., signaling by mechanistic target

of rapamycin) and the involvement of 288 lncRNAs in the *cis*-regulation of their sense-overlapping genes. Interestingly, *Rb1* regulation by the lncRNA NONMMUT021591s was predicted; 60 transcription factors regulating expression of 372 lncRNAs predicted the regulation of the lncRNA, FR015378, by C/EBPβ (54). Additional studies could determine the biological contribution of lncRNAs in modulating the function and differentiation of MDSCs in the context of infections.

CONCLUSIONS AND FUTURE PERSPECTIVES

Studies have suggested that specific lncRNAs control the differentiation of MDSC subsets and immunosuppressive function. Furthermore, lncRNAs show specific patterns of expression depending on the cell and tissue types (39). Thus, lncRNAs could be potential specific markers of MDSC subsets and several MDSCs with diverse phenotypes that have been observed in various diseases, but these have yet to be determined. In addition, controversy has been generated because the close relationship among MDSCs, neutrophils, and monocytes. Analyzing the expression profile of lncRNAs in MDSCs compared with that of lncRNAs in myeloid cells could provide new insights into the differences described among these cell types. Studies analyzing lncRNAs exclusively in mouse MDSCs should be viewed with caution, considering that lncRNAs seem to be poorly conserved among various species (56). Therefore, investigations into the modulation of MDSC activity by lncRNAs should consider the evolutionary conservation of lncRNA in human MDSCs for potential applications against human diseases. The lncRNA expression profile in human MDSCs has yet to be reported.

Understanding the regulation of immunosuppressive function and the accumulation of MDSCs to find therapeutic targets that modulate immunosuppression is more important than the classification of MDSCs among myeloid cells. The lncRNAs regulate the activity of different transcription factors (e.g., C/EBPβ, CHOP, and RUNX1) involved in the differentiation and suppression of MDSCs. Thus, these non-coding RNAs might play significant roles in the two-signal model (11), in which lncRNAs (such as Olfr29-ps1, lnc-CHOP, RNCR3, and RUNXOR) could participate in the first phase during expansion of the MDSCs, and then in the second phase to promote MDSC activation. The Pvt1 could participate only in the second phase. The versatility of lncRNAs in the recognition of different targets could facilitate their participation in both phases. In addition, the factors produced by tumors, hypoxia, or an inflammatory microenvironment could support the expression of some non-coding RNAs. Moreover, the chronic and low-dose stimuli generated by inflammatory and tumoral factors could promote the downregulation of lncRNAs that inhibit the accumulation or immunosuppressive function of MDSCs. Future studies will determine whether this perception is correct.

In addition to intracellular regulation, lncRNAs can exert intercellular effects *via* exosomes (57, 58). These extracellular nanovesicles are derived from endosomes, have a diameter of 30–100 nm, and are secreted by different cell types, including cells with a myeloid lineage, such as MDSCs (59, 60). Limited information on MDSC exosomes indicates that they can exert effects associated with immunosuppression and the promotion of tumorigenesis (61). The MDSC exosomes carry proteins, RNAs, and miRNAs (62), but the presence of lncRNAs in MDSC exosomes has not been investigated. Future studies addressing this issue would be important, because lncRNAs are supposedly selectively packaged in exosomes and secreted by cancer cells and stroma cells to modulate the growth, metastasis, angiogenesis, and chemoresistance of cancer cells (57, 58). Moreover, the characterization of lncRNAs in exosomes secreted by myeloid lineage cells is not widely understood.

The lncRNAs involved in the biological behavior of MDSCs could facilitate the development of novel therapeutic approaches. However, if these lncRNAs are involved in multiple physiological functions or have contrasting effects in different cell types, then alteration/manipulation of lncRNAs could also generate undesirable side effects. Thus, one cannot suggest that targeting lncRNAs is feasible or practical. A more comprehensive understanding of lncRNA functions and the molecular mechanisms implicated in the modulation of MDSCs is necessary.

The central role of MDSCs in generating immunosuppressive tumor microenvironments supports the growth and progression of tumor cells. In addition, a general understanding of the modulation of the inflammatory response by MDSCs in other diseases has been improved in recent years. Thus, molecules that regulate the biological behavior of MDSCs could be the targets of therapies against these diseases.

The lncRNAs are involved in the control of MDSC differentiation and immunosuppressive programs in cancer via various molecular mechanisms (**Table 1**). Nevertheless, the functional link between some lncRNAs and MDSCs does not seem to be sufficiently strong. Thus, the study of the mechanisms by which lncRNAs modulate MDSCs is in its infancy. The lncRNAs control gene expression and diverse biological functions in health and disease in both cell- and tissue-specific manners. Hence, future studies should aim to identify the novel lncRNAs that regulate MDSC activity, so they can be applied in immunomodulatory therapy or as biomarkers.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer.* (2019) 120:16–25. doi: 10.1038/s41416-018-0333-1
2. Budhwar S, Verma P, Verma R, Rai S, Singh K. The Yin and Yang of myeloid derived suppressor cells. *Front Immunol.* (2018) 9:2776. doi: 10.3389/fimmu.2018.02776
3. Ma H, Xia CQ. Phenotypic and functional diversities of myeloid-derived suppressor cells in autoimmune diseases. *Mediators Inflamm.* (2018) 2018:4316584. doi: 10.1155/2018/4316584
4. Penaloza HF, Alvarez D, Munoz-Durango N, Schultz BM, Gonzalez PA, Kalergis AM, et al. The role of myeloid-derived suppressor cells in chronic infectious diseases and the current methodology available for their study. *J Leukoc Biol.* (2019) 105:857–72. doi: 10.1002/JLB.MR0618-233R
5. Safari E, Ghorghanlu S, Ahmadi-Khiavi H, Mehranfar S, Rezaei R, Motallebnezhad M. Myeloid-derived suppressor cells and tumor: current knowledge and future perspectives. *J Cell Physiol.* (2019) 234:9966–81. doi: 10.1002/jcp.27923
6. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann N Y Acad Sci.* (2014) 1319:47–65. doi: 10.1111/nyas.12469
7. Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. *Oncotarget.* (2017) 8:3649–65. doi: 10.18632/oncotarget.12278
8. Salminen A, Kaarniranta K, Kauppinen A. Immunosenescence: the potential role of myeloid-derived suppressor cells (MDSC) in age-related immune deficiency. *Cell Mol Life Sci.* (2019) 76:1901–18. doi: 10.1007/s00018-019-03048-x
9. Chiba Y, Mizoguchi I, Hasegawa H, Ohashi M, Orii N, Nagai T, et al. Regulation of myelopoiesis by proinflammatory cytokines in infectious diseases. *Cell Mol Life Sci.* (2018) 75:1363–76. doi: 10.1007/s00018-017-2724-5
10. Consonni FM, Porta C, Marino A, Pandolfo C, Mola S, Bleve A, et al. Myeloid-derived suppressor cells: ductile targets in disease. *Front Immunol.* (2019) 10:949. doi: 10.3389/fimmu.2019.00949
11. Condamine T, Mastio J, Gabrilovich DI. Transcriptional regulation of myeloid-derived suppressor cells. *J Leukoc Biol.* (2015) 98:913–22. doi: 10.1189/jlb.4RI0515-204R
12. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res.* (2017) 5:3–8. doi: 10.1158/2326-6066.CIR-16-0297
13. Hirai H, Yokota A, Tamura A, Sato A, Maekawa T. Non-steady-state hematopoiesis regulated by the C/EBPbeta transcription factor. *Cancer Sci.* (2015) 106:797–802. doi: 10.1111/cas.12690
14. Marigo I, Bosio E, Solito S, Mesa C, Fernandez A, Dolcetti L, et al. Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. *Immunity.* (2010) 32:790–802. doi: 10.1016/j.immuni.2010.05.010
15. Hirai H, Zhang P, Dayaram T, Hetherington CJ, Mizuno S, Imanishi J, et al. C/EBPbeta is required for 'emergency' granulopoiesis. *Nat Immunol.* (2006) 7:732–9. doi: 10.1038/ni1354
16. McPeak MB, Youssef D, Williams DA, Pritchett CL, Yao ZQ, McCall CE, et al. Frontline science: myeloid cell-specific deletion of Cebpb decreases sepsis-induced immunosuppression in mice. *J Leukoc Biol.* (2017) 102:191–200. doi: 10.1189/jlb.4HI1216-537R
17. Gao Y, Wang T, Li Y, Zhang Y, Yang R. Lnc-chop promotes immunosuppressive function of myeloid-derived suppressor cells in tumor and inflammatory environments. *J Immunol.* (2018) 200:2603–14. doi: 10.4049/jimmunol.1701721
18. Gao Y, Sun W, Shang W, Li Y, Zhang D, Wang T, et al. Lnc-C/EBPbeta negatively regulates the suppressive function of myeloid-derived suppressor cells. *Cancer Immunol Res.* (2018) 6:1352–63. doi: 10.1158/2326-6066.CIR-18-0108
19. Tesi RJ. MDSC; the most important cell you have never heard of. *Trends Pharmacol Sci.* (2019) 40:4–7. doi: 10.1016/j.tips.2018.10.008
20. Zhang C, Wang S, Liu Y, Yang C. Epigenetics in myeloid derived suppressor cells: a sheathed sword towards cancer. *Oncotarget.* (2016) 7:57452–63. doi: 10.18632/oncotarget.10767
21. Sandoval-Basilio J, Gonzalez-Gonzalez R, Bologna-Molina R, Isiordia-Espinoza M, Leija-Montoya G, Alcaraz-Estrada SL, et al. Epigenetic mechanisms in odontogenic tumors: a literature review. *Arch Oral Biol.* (2018) 87:211–7. doi: 10.1016/j.archoralbio.2017.12.029
22. El Gazzar M. microRNAs as potential regulators of myeloid-derived suppressor cell expansion. *Innate Immun.* (2014) 20:227–38. doi: 10.1177/1753425913489850
23. Shang W, Tang Z, Gao Y, Qi H, Su X, Zhang Y, et al. LncRNA RNCR3 promotes chop expression by sponging miR-185-5p during MDSC differentiation. *Oncotarget.* (2017) 8:111754–69. doi: 10.18632/oncotarget.22906
24. Shang W, Gao Y, Tang Z, Zhang Y, Yang R. The pseudogene Olfr29-ps1 promotes the suppressive function and differentiation of monocytic MDSCs. *Cancer Immunol Res.* (2019) 7:813–27. doi: 10.1158/2326-6066.CIR-18-0443
25. Zheng Y, Tian X, Wang T, Xia X, Cao F, Tian J, et al. Long noncoding RNA Pvt1 regulates the immunosuppression activity of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Mol Cancer.* (2019) 18:61. doi: 10.1186/s12943-019-0978-2
26. Zhou Q, Tang X, Tian X, Tian J, Zhang Y, Ma J, et al. LncRNA MALAT1 negatively regulates MDSCs in patients with lung cancer. *J Cancer.* (2018) 9:2436–42. doi: 10.7150/jca.24796
27. Tian X, Ma J, Wang T, Tian J, Zhang Y, Mao L, et al. Long non-coding RNA HOXA transcript antisense RNA myeloid-specific 1-HOXA1 axis downregulates the immunosuppressive activity of myeloid-derived suppressor cells in lung cancer. *Front Immunol.* (2018) 9:473. doi: 10.3389/fimmu.2018.00473
28. Tian X, Ma J, Wang T, Tian J, Zheng Y, Peng R, et al. Long non-coding RNA RUNXOR accelerates MDSC-mediated immunosuppression in lung cancer. *BMC Cancer.* (2018) 18:660. doi: 10.1186/s12885-018-4564-6
29. Ma X, Sheng S, Wu J, Jiang Y, Gao X, Cen X, et al. LncRNAs as an intermediate in HPV16 promoting myeloid-derived suppressor cell recruitment of head and neck squamous cell carcinoma. *Oncotarget.* (2017) 8:42061–75. doi: 10.18632/oncotarget.14939
30. Fujisaka Y, Iwata T, Tamai K, Nakamura M, Mochizuki M, Shibuya R, et al. Long non-coding RNA HOTAIR up-regulates chemokine (C-C motif) ligand 2 and promotes proliferation of macrophages and myeloid-derived suppressor cells in hepatocellular carcinoma cell lines. *Oncol Lett.* (2018) 15:509–14. doi: 10.3892/ol.2017.7322
31. Wei S, Wang K. Long noncoding RNAs: pivotal regulators in acute myeloid leukemia. *Exp Hematol Oncol.* (2015) 5:30. doi: 10.1186/s40164-016-0059-9
32. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. *Cancer Res.* (2017) 77:3965–81. doi: 10.1158/0008-5472.CAN-16-2634
33. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet.* (2016) 17:47–62. doi: 10.1038/nrg.2015.10
34. Ayupe AC, Tahira AC, Camargo L, Beckedorff FC, Verjovski-Almeida S, Reis EM. Global analysis of biogenesis, stability and sub-cellular localization of lncRNAs mapping to intragenic regions of the human genome. *RNA Biol.* (2015) 12:877–92. doi: 10.1080/15476286.2015.1062960
35. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* (2011) 25:1915–27. doi: 10.1101/gad.17446611
36. Marchese FP, Raimondi I, Huarte M. The multidimensional mechanisms of long noncoding RNA function. *Genome Biol.* (2017) 18:206. doi: 10.1186/s13059-017-1348-2
37. Anfossi S, Babayan A, Pantel K, Calin GA. Clinical utility of circulating non-coding RNAs–an update. *Nat Rev Clin Oncol.* (2018) 15:541–63. doi: 10.1038/s41571-018-0035-x
38. Balas MM, Johnson AM. Exploring the mechanisms behind long noncoding RNAs and cancer. *Noncoding RNA Res.* (2018) 3:108–17. doi: 10.1016/j.ncrna.2018.03.001
39. Mathy NW, Chen XM. Long non-coding RNAs (lncRNAs) and their transcriptional control of inflammatory responses. *J Biol Chem.* (2017) 292:12375–82. doi: 10.1074/jbc.R116.760884
40. Tian X, Tian J, Tang X, Ma J, Wang S. Long non-coding RNAs in the regulation of myeloid cells. *J Hematol Oncol.* (2016) 9:99. doi: 10.1186/s13045-016-0333-7

41. Zhang L, Cao Y, Wei M, Jiang X, Jia D. Long noncoding RNA-RNCR3 overexpression deleteriously affects the growth of glioblastoma cells through miR-185-5p/Kruppel-like factor 16 axis. *J Cell Biochem.* (2018) 119:9081–9. doi: 10.1002/jcb.27167
42. Shan K, Jiang Q, Wang XQ, Wang YN, Yang H, Yao MD, et al. Role of long non-coding RNA-RNCR3 in atherosclerosis-related vascular dysfunction. *Cell Death Dis.* (2016) 7:e2248. doi: 10.1038/cddis.2016.145
43. Shan K, Li CP, Liu C, Liu X, Yan B. RNCR3: a regulator of diabetes mellitus-related retinal microvascular dysfunction. *Biochem Biophys Res Commun.* (2017) 482:777–83. doi: 10.1016/j.bbrc.2016.11.110
44. Tian C, Deng Y, Jin Y, Shi S, Bi H. Long non-coding RNA RNCR3 promotes prostate cancer progression through targeting miR-185-5p. *Am J Transl Res.* (2018) 10:1562–70.
45. Thevenot PT, Sierra RA, Raber PL, Al-Khami AA, Trillo-Tinoco J, Zarreii P, et al. The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. *Immunity.* (2014) 41:389–401. doi: 10.1016/j.immuni.2014.08.015
46. Colombo T, Farina L, Macino G, Paci P. PVT1: a rising star among oncogenic long noncoding RNAs. *Biomed Res Int.* (2015) 2015:304208. doi: 10.1155/2015/304208
47. Lu D, Luo P, Wang Q, Ye Y, Wang B. lncRNA PVT1 in cancer: a review and meta-analysis. *Clin Chim Acta.* (2017) 474:1–7. doi: 10.1016/j.cca.2017.08.038
48. Zhao M, Wang S, Li Q, Ji Q, Guo P, Liu X. MALAT1: a long non-coding RNA highly associated with human cancers. *Oncol Lett.* (2018) 16:19–26. doi: 10.3892/ol.2018.8613
49. Wan L, Kong J, Tang J, Wu Y, Xu E, Lai M, et al. HOTAIRM1 as a potential biomarker for diagnosis of colorectal cancer functions the role in the tumour suppressor. *J Cell Mol Med.* (2016) 20:2036–44. doi: 10.1111/jcmm.12892
50. Xin J, Li J, Feng Y, Wang L, Zhang Y, Yang R. Downregulation of long noncoding RNA HOTAIRM1 promotes monocyte/dendritic cell differentiation through competitively binding to endogenous miR-3960. *Onco Targets Ther.* (2017) 10:1307–15. doi: 10.2147/OTT.S124201
51. Wang H, Li W, Guo R, Sun J, Cui J, Wang G, et al. An intragenic long noncoding RNA interacts epigenetically with the RUNX1 promoter and enhancer chromatin DNA in hematopoietic malignancies. *Int J Cancer.* (2014) 135:2783–94. doi: 10.1002/ijc.28922
52. Tian J, Rui K, Tang X, Ma J, Wang Y, Tian X, et al. MicroRNA-9 regulates the differentiation and function of myeloid-derived suppressor cells via targeting Runx1. *J Immunol.* (2015) 195:1301–11. doi: 10.4049/jimmunol.1500209
53. Tang Q, Hann SS. HOTAIR: an oncogenic long non-coding RNA in human cancer. *Cell Physiol Biochem.* (2018) 47:893–913. doi: 10.1159/000490131
54. Yu A, Wang Y, Yin J, Zhang J, Cao S, Cao J, et al. Microarray analysis of long non-coding RNA expression profiles in monocytic myeloid-derived suppressor cells in Echinococcus granulosus-infected mice. *Parasit Vectors.* (2018) 11:327. doi: 10.1186/s13071-018-2905-6
55. Pan W, Zhou HJ, Shen YJ, Wang Y, Xu YX, Hu Y, et al. Surveillance on the status of immune cells after Echinnococcus granulosus protoscoleces infection in Balb/c mice. *PLoS ONE.* (2013) 8:e59746. doi: 10.1371/journal.pone.0059746
56. Leti F, DiStefano JK. Long noncoding RNAs as diagnostic and therapeutic targets in type 2 diabetes and related complications. *Genes (Basel).* (2017) 8:E207. doi: 10.3390/genes8080207
57. Sun Z, Yang S, Zhou Q, Wang G, Song J, Li Z, et al. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. *Mol Cancer.* (2018) 17:82. doi: 10.1186/s12943-018-0831-z
58. Wang M, Zhou L, Yu F, Zhang Y, Li P, Wang K. The functional roles of exosomal long non-coding RNAs in cancer. *Cell Mol Life Sci.* (2019) 76:2059–76. doi: 10.1007/s00018-019-03018-3
59. Zoller M. Janus-faced myeloid-derived suppressor cell exosomes for the good and the bad in cancer and autoimmune disease. *Front Immunol.* (2018) 9:137. doi: 10.3389/fimmu.2018.00137
60. Wu R, Gao W, Yao K, Ge J. Roles of exosomes derived from immune cells in cardiovascular diseases. *Front Immunol.* (2019) 10:648. doi: 10.3389/fimmu.2019.00648
61. Shen M, Ren X. New insights into the biological impacts of immune cell-derived exosomes within the tumor environment. *Cancer Lett.* (2018) 431:115–22. doi: 10.1016/j.canlet.2018.05.040
62. Geis-Asteggiante L, Belew AT, Clements VK, Edwards NJ, Ostrand-Rosenberg S, El-Sayed NM, et al. Differential content of proteins, mRNAs, and miRNAs suggests that MDSC and their exosomes may mediate distinct immune suppressive functions. *J Proteome Res.* (2018) 17:486–98. doi: 10.1021/acs.jproteome.7b00646

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