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Location, location, location: functional and phenotypic heterogeneity between tumor-infiltrating and non-infiltrating myeloid-derived suppressor cells

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Abbreviations: ATRA, all-trans retinoic acid; Bv8, Bombina variagata peptide 8; CTLA-4, cytotoxic T-lymphocyte antigen-4; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; IL-4Rα, interleukin-4 receptor alpha; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; M-CSF, macrophage-colony stimulating factor; MDSCs, myeloid-derived suppressor cells; NS cells, natural suppressor cells; PD-L1, programmed death-ligand 1; PHA, phytohemagglutinin; ROS, reactive oxygen species; siRNA, small interfering ribonucleic acid; TAMs, tumor-associated macrophages; T_{reg}, regulatory T cells; VEGF, vascular endothelial growth factor.

An increasing number of studies is focusing on the role of myeloid-derived suppressor cells (MDSCs) in the suppression of antitumor immune responses. Although the main site of action for MDSCs is most likely the tumor microenvironment, the study of these cells has been largely restricted to MDSCs derived from peripheral lymphoid organs. Only in a minority of studies MDSCs isolated from the tumor microenvironment have been characterized. This review will give an overview of the data available on the phenotypical and functional differences between tumor-derived MDSCs and MDSCs isolated from the spleen of tumor-bearing mice or from the peripheral blood of cancer patients.

Introduction

The link between inflammation and tumor progression is longstanding. Already in 1863 Virchow observed that neoplastic tissue is infiltrated by leukocytes,[1] giving rise to the hypothesis that cancer arises at sites of chronic inflammation. Under normal physiological conditions, inflammation is a self-limiting process, but dysfunctions in one of the inflammatory pathways can lead to pathogenesis and eventually to tumorigenesis.[2] Despite the fact that our immune system is able to recognize and eliminate tumor cells, many tumors can escape immune control by various mechanisms. The presence of several subsets of suppressive immune cells, including regulatory T cells (T_{reg}), tumor-associated macrophages (TAMs), and MDSCs, contribute to the immunosuppressive microenvironment[3] and help tumors escape immune control. Already in the late 1970s different research groups described cells of myeloid origin that had the capacity to inhibit T-cell responses. These cells were termed natural suppressor (NS) cells,[4,5] but because of technical and experimental limitations it was very difficult to fully characterize the phenotype and exact function of these cells. It was only in the late 1990s that two groups independently rediscovered these cells[6,7] and since then the interest in immunosuppressive cells of myeloid origin has steadily increased. From then on, these cells were called "immature myeloid cells." Since this term reflects only the origin of the cells and does not emphasize the most important characteristic of these cells, namely their ability to suppress immune responses, a consensus was reached to call these cells "myeloid-derived suppressor cells."[8,9] The increased interest in these cells is reflected by the fact that in 2013 over 300 research articles were published on this topic. Moreover, many research groups are developing strategies to specifically target these MDSCs in order to improve antitumor immune responses, further emphasizing the importance of these cells in the field of tumor immunology. These targeted strategies include blocking the differentiation and accumulation of MDSCs at the tumor site, blocking their expansion and interfering with their function. These strategies have been extensively reviewed in ref.[10], and will, therefore, not be further addressed in this review. More and more evidence shows that MDSCs display a high phenotypic plasticity and can, under the influence of cytokines such as interleukin 12 (IL-12) and interferon-gamma (IFN-γ), even acquire characteristics of antigen-presenting cells.[6,11] In contrast, all-trans retinoic acid (ATRA) was shown to trigger the differentiation of Gr-1$^+$ cells into mature F4/80$^+$ macrophages which were more potent immune suppressors on a per-cell basis. These data highlight the potent immunosuppressive functions of macrophages and support the development of therapeutic strategies to enhance antitumor immunity by targeting inhibitory myeloid cells as a collective

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group.[12] Moreover, in analogy to the M1/M2 polarization in macrophages, MDSCs also show an M1/M2 classification in the tumor microenvironment. Different studies have shown that MDSCs present within the tumor microenvironment exhibit M2 characteristics, which accelerates tumor growth which is mediated by enhanced arginase activity, an increased secretion of immunosuppressive cytokines and the induction of angiogenesis.[13,14] However, Umemura et al. showed that tumor-infiltrating MDSCs are pleiotropic-inflamed macrophages that can simultaneously display both M1- and M2-characteristics.[15] In contrast, it has been shown that M2-type MDSCs can be skewed toward M1-type cells by lipopolysaccharide (LPS) through the p38 mitogen-activated protein kinases (MAPK) pathway,[16] further underlining the plasticity of these cells. Thus, it is clear that MDSCs display a high degree of plasticity and that their exact fate will be determined by various factors inherent to the tumor type.

Murine MDSCs

Phenotype

MDSCs represent a heterogeneous population of myeloid cells that share some phenotypic characteristics with monocytes, macrophages, dendritic cells, and granulocytes,[17] but can be distinguished from these cells by their potent immunosuppressive activities. The differentiation and accumulation of these MDSCs in tumor-bearing hosts is driven by different factors, such as IL-6,[18] vascular endothelial growth factor (VEGF),[19,20] granulocyte-macrophage colony-stimulating factor (GM-CSF),[21] IL-1β,[22] prostaglandin E_2 (PGE_2),[23] S100A8/A9 proteins[24] and hypoxia.[25] These cells can express different surface markers, depending on the tumor type, disease stage, factors released by the tumors and their anatomical location. In mice, the markers CD11b and Gr-1 define this immunosuppressive cell population. Antibodies that specifically recognize Gr-1 bind to two separate antigens, Ly6G and Ly6C.[26] The use of epitope specific antibodies together with morphological analysis has led to the identification of two functionally distinct subtypes of MDSCs: $CD11b^{+}Ly6G^{+}Ly6C^{low}$ MDSCs, which are morphologically similar to polymorphonuclear granulocytes, whereas $CD11b^{+}Ly6G^{-}Ly6C^{high}$ MDSCs have a monocytic morphology.[27] Both subsets can suppress T-cell proliferation, although they use different mechanisms to exert their function and various reports indicate that these two populations might have distinct functions in infectious diseases, autoimmune diseases, graft-versus-host disease, and cancer.[28-31]

Youn et al. were the first to perform a broad phenotypical and functional analysis of the two subtypes of MDSCs in 10 different models of lung cancer, breast cancer, colon cancer, melanoma, and sarcoma developed in three different strains of mice. Unfortunately, they only focused on spleen-derived MDSCs and they did not extend their findings to MDSCs present within the tumor microenvironment. In all tumor models studied, the number of MDSCs was significantly increased compared to that in naïve mice. However, the extent of MDSC expansion varied between different tumor models. Moreover, in most of the tumor models studied, it were predominantly the $CD11b^{+}Ly6G^{+}Ly6C^{low}$ granulocytic MDSCs that expanded, except for the EL4 tumor model where both subsets were expanded equally.[27,31] In a recent study, the same group showed that in tumor-bearing mice, monocytic MDSCs differentiate toward granulocytic MDSCs through the epigenetic silencing of the gene encoding retinoblastoma, a transcriptional regulator that controls cellular proliferation and differentiation. Thus, although it was previously assumed that monocytic and granulocytic MDSCs develop along a different pathway, this study suggests that, at least in cancer, MDSC development is altered, resulting in the conversion of monocytic MDSCs toward granulocytic MDSCs.[32] However, more research will be required to fully elucidate the mechanisms and the tumor-derived factors that determine the fate of MDSCs and dictate the relationship between the different subsets of MDSCs. A number of different surface molecules implicated in the suppressive function of MDSCs have been described. The Interleukin-4 receptor α (IL-4Rα) has been reported to be essential for the development and function of MDSCs.[33,34] However, these studies used *in vitro* generated MDSCs, so the relevance for primary MDSCs, either derived from the spleen or from the tumor, remains unclear. In contrast, other studies, using MDSCs isolated from the spleen of tumor-bearing animals and from IL-4Rα knock-out mice, demonstrated that the IL-4Rα is not essential for the accumulation and function of MDSCs.[23,27,35] Unfortunately, none of these studies looked at the expression and function of the IL-4Rα on MDSCs derived from within the tumor microenvironment. Members of the B7-family were shown to be directly involved in the suppression of immune responses.[36,37] CD80 (B7-1), expressed on antigen-presenting cells, has the ability to bind both to a stimulatory receptor, CD28, and to an inhibitory receptor CD152 (also known as CTLA-4, cytotoxic T-lymphocyte antigen-4) on T cells. Moreover, CD80 can also bind to programmed death-ligand 1 (PD-L1), which results in the delivery of inhibitory signals to T cells.[38-40] Depending on these interactions an immune response will be evoked or dampened. The complexity of these ligand-receptor interactions hampers the study of the role of these molecules in the suppressive function of MDSCs and many conflicting results have been published. In their search for additional markers for MDSCs, Youn et al. did not observe higher levels of CD80, PD-L1, and PD-L2 on MDSCs isolated from the spleen of tumor-bearing animals compared to the expression levels found on immature myeloid cells isolated from the spleen of naïve animals. However, they did not look at the expression of these markers on MDSCs isolated from the tumor microenvironment.[27] In a recent study Noman et al. showed that MDSCs at the tumor site had a higher expression of PD-L1 compared to MDSCs isolated from the spleen. PD-L1 is upregulated on these MDSCs under the influence of hypoxia and blockade of PD-L1 under hypoxic conditions abrogated the MDSC-mediated T-cell suppression by modulating MDSC cytokine production.[41] In different tumor models it has been shown that CD80 expression is upregulated on tumor-derived MDSCs.[42-45] Moreover in some tumor models CD80 expression was also elevated on spleen-derived MDSCs.[24,42] These

conflicting observations could be explained by the use of different tumor models (subcutaneously implanted tumors vs. tumors grown as ascites), the disease stage at which CD80 expression was evaluated and the subset of MDSCs under investigation. Conflicting results have been published about the role of this upregulated CD80 expression in the suppressive function of the MDSCs, which will be discussed further. Because of the myeloid origin of MDSCs, CD115 (macrophage-colony stimulating factor (M-CSF) receptor) and F4/80 are two additional markers used to further characterize MDSCs. It has been claimed that Gr-1 and CD115 may be better markers to define MDSCs compared to Gr-1 and CD11b.[46] However, Youn et al. only found higher levels of CD115 on MDSCs in 2 out of 10 tested tumor models.[27] Moreover, it has been shown in three different tumor models, including the EL4 thymoma, the B16.F10 melanoma, and the CC10 spontaneous lung tumor model, that only a minority of MDSCs express CD115 or F4/80. However, the expression of both molecules is slightly higher on tumor-associated MDSCs compared to spleen-derived MDSCs, although these differences were not statistically significant.[25] One should be cautious when interpreting these data. Since protocols for isolating MDSCs vary extensively, it cannot be ruled out that these $F4/80^+$ cells are actually contaminating TAMs rather than MDSCs. TAMs are a cellular population that can be histologically confused with MDSCs, but that are defined as mature and fully differentiated macrophages.[47] However, distinguishing TAMs from MDSCs can be technically challenging. Gene expression profiling of $CD11b^+Gr\text{-}1^+$ cells from the spleens and tumors of 4T1 tumor-bearing mice revealed that genes involved in extracellular matrix remodeling, immunomodulation and hypoxia regulation were markedly upregulated in tumor-derived cells compared to their non-infiltrating counterparts.[48] However, the functional importance of these upregulated genes in the function of tumor-derived MDSCs has so far not been determined.

Function

Only in a minority of studies MDSCs isolated from the tumor microenvironment have been fully characterized. In most of these studies, tumors are grown as ascites in order to facilitate the isolation of tumor-derived MDSCs. In order to directly compare the suppressive function of MDSCs from the tumor site to MDSCs from the spleen Corzo et al. developed a model where EL4 tumors were grown as ascites.[25] They evaluated the effect of spleen-derived and tumor-derived MDSCs on the IFN-γ production and T-cell proliferation in both an antigen-specific and non-specific system. Both spleen- and tumor-derived MDSCs were able to suppress antigen-specific T-cell responses, although the level of suppression was significantly higher in tumor-derived MDSCs. However, the major differences were observed in a non-antigen specific setting, where spleen-derived MDSCs did not suppress T-cell responses while MDSCs isolated from the tumor exerted a profound suppressive effect on these T cells.[25] This is in contrast with the data obtained by Haverkamp et al. who showed, in a model of acute and chronic prostate inflammation, that MDSCs derived from the spleen were not functional whereas cells isolated from the inflammatory site were able to inhibit T-cell proliferation and expressed higher levels of arginase and inducible nitric oxide synthase (iNOS).[43] The difference between these 2 studies is the duration of the proliferation assay: 72 h in the experiments performed by Corzo et al. vs. only 12 h in the experiments of Haverkamp et al. The latter wanted to minimize the exposure of the MDSCs to IFNγ because it has been shown that IFNγ, produced during a standard proliferation assay of 3 d, converts precursor splenic $CD11b^+Gr\text{-}1^+$ cells (without suppressive activity) into fully functional MDSCs.[43,49] However, we have shown in a non-antigen specific system, that spleen-derived MDSCs have the ability to suppress T-cell proliferation and cytokine secretion, although to a lower extent than the tumor-derived MDSCs. Moreover, we have shown that there is no production of IFN-γ when spleen-derived $CD11b^+Ly6G^+Ly6C^{int}$ MDSCs were cocultured with splenocytes, indicating that at least for the granulocytic subset of MDSCs other mechanisms are responsible for the suppression of T-cell proliferation by spleen-derived MDSCs.[45] Conflicting results concerning the suppressive activity of spleen- and tumor-derived MDSCs could be explained by differences in the activation status of the responder cells, differences in the duration of the suppression assay, differences between antigen-specific and antigen non-specific T-cell responses, differences in the subsets of MDSCs under investigation and the use of different tumor models.[24,25,27,43,49]

Different factors and mechanisms are involved in the suppressive activity of MDSCs, including arginase 1 activity, iNOS, the induction of T_{reg}, downregulation of the T-cell receptor, etc. These mechanisms have been reviewed in detail elsewhere and will not be the subject of this review.[50-52] Here, we will focus on the differences in the mechanisms used by spleen- or tumor-derived MDSCs to exert their function (**Fig. 1**). Several groups have shown an increased activity for both arginase and iNOS and a higher production of nitrite (NO_2^-) in tumor-derived MDSCs compared to their non-infiltrating counterparts. In contrast, higher amounts of reactive oxygen species (ROS) were detected in spleen-derived MDSCs.[25,45,53] These data indicate that MDSCs isolated from either the spleen or the tumor microenvironment use different mechanisms to exert their function, which can explain the differences in suppressive strength between spleen- and tumor-derived MDSCs. However, further research is needed to fully elucidate these differences and their importance for the suppressive function of MDSCs.

The importance of the CD80-CD28/CTLA-4 pathway in the suppressive function of MDSCs is not completely clear. As mentioned previously, CD80 expression is upregulated on tumor-derived MDSCs,[42-45] as well as on spleen-derived MDSCs in some tumor models.[24,42] The use of CD80-specific neutralizing antibodies or small interfering ribonucleic acid (siRNA) against CD80 was shown to partially inhibit the suppressive function of MDSCs, indicating that CD80 does play a role in the suppressive function of MDSCs but that other factors are also involved.[42,45] Moreover, when mouse ovarian surface epithelial cell line (MOSEC) ID8 cells were injected subcutaneously into $CD80^{-/-}$ mice a slower tumor growth associated with a decreased suppressive potential of ovarian carcinoma-associated MDSCs was observed, compared to

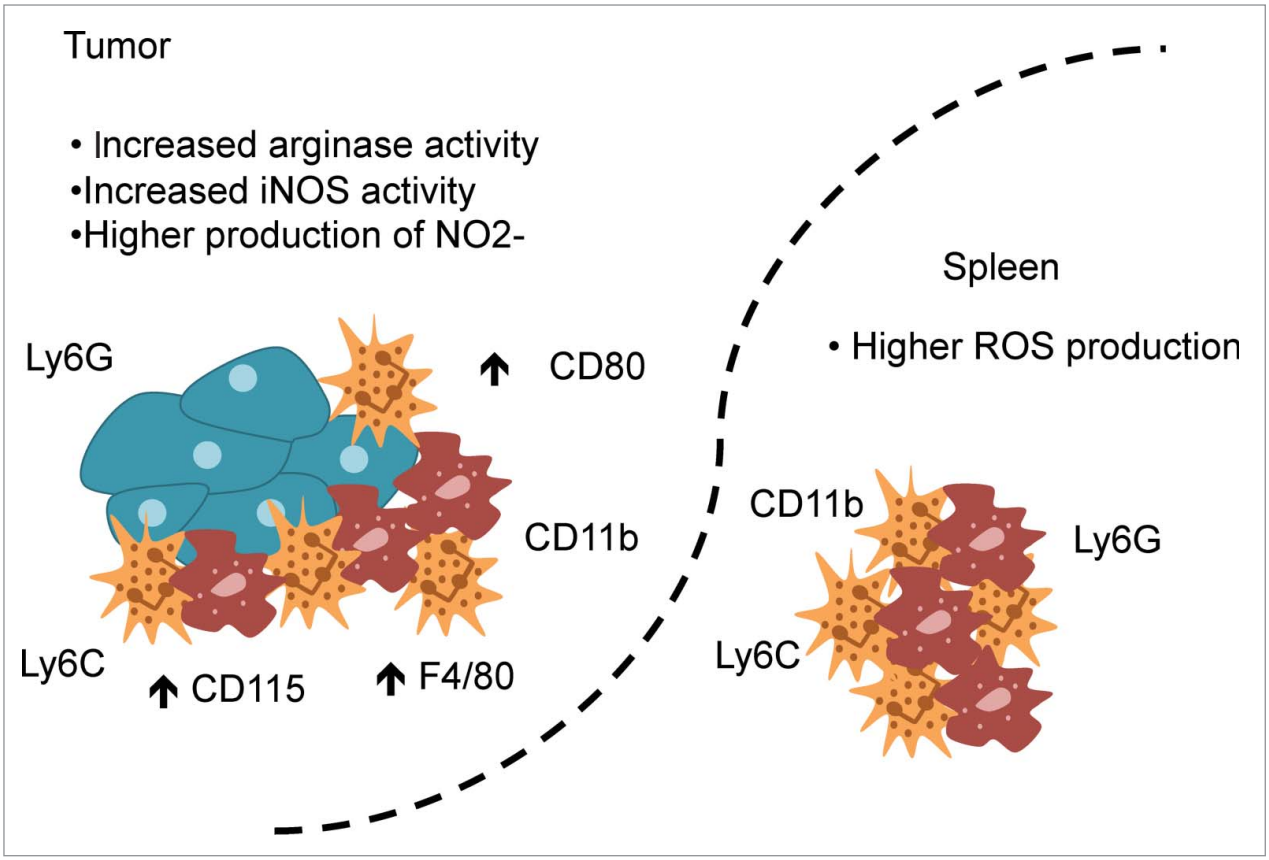

Figure 1. MDSCs present in the tumor use different mechanisms to exert their suppressive function compared to their peripheral counterparts. Both spleen- and tumor-derived MDSCs express the markers CD11b, Ly6G, and Ly6C but MDSCs derived from the tumor express higher levels of CD115, F4/80 and CD80. An increased arginase activity, an increased inducible nitric oxide synthase (iNOS) activity, and a higher production of nitrite (NO_2^-) were observed in tumor-derived MDSCs, while spleen-derived MDSCs produced higher amounts of reactive oxygen species (ROS).

$CD80^{+/+}$ mice.[42] In contrast, Tomihara et al. showed that MDSCs derived from ascites of ovarian tumor-bearing mice are rather immunostimulatory and augment the proliferation of cytotoxic T cells via signaling through CD80.[54] In order to fully understand these conflicting results, a consensus about the phenotype of MDSCs and the type of assays that have to be performed to evaluate their suppressive function has to be reached.

Human MDSCs

Phenotype

In humans, $CD34^+$ cells that accumulate under the influence of GM-CSF and have the capacity to inhibit the secretion of IL-2 by activated lymphocytes, and, thus, possess MDSC-like properties, were described for the first time in patients with head and neck cancer.[55] Human MDSCs have so far been inadequately characterized because of the lack of uniform markers (such as Gr-1 in mice), and a unified phenotype. The lack of defined and homogeneous markers for MDSCs in humans hampers the study of the biological function and clinical impact of these MDSCs in cancer patients. In accordance with the data obtained in murine models, attempts have been made to divide human MDSCs in two subtypes: a more granulocytic and a more monocytic type of cell. It has been shown that both subsets express the common myeloid markers CD11b and CD33 but lack the expression of mature myeloid markers. Moreover, the monocytic cells express CD14 while the granulocytic cells express CD15. Many laboratories are trying to further characterize different subsets of MDSCs in different types of cancer using different sets and combinations of markers. A comprehensive overview of the different phenotypes of MDSCs in different types of cancer can be found in ref[56]. These findings are summarized in **Table 1**. Whether this broad variety in described phenotypes can be attributed to different mechanisms of induction/expansion of MDSCs in different types of cancer or whether this is simply due to the variation in markers used by different research groups, is not clear yet. The variation in expression of different cell surface markers on MDSCs derived from patients with different types of cancer indicates the existence of subpopulations of MDSCs, as this is seen in different mouse models as well. This is not surprising since MDSCs accumulate under the influence of tumor-derived factors. Different types of tumors can secrete distinct sets of inflammatory molecules, which will lead to the accumulation of MDSCs with a particular and perhaps even unique phenotype. However, given the difficulties to reach the tumor and to obtain

Table 1. Phenotype of MDSCs described in different cancer types

Cancer type	SSC	CD3	CD1a	CD11c	CD11b	CD13	CD14	CD15	CD16	CD19	CD20	CD24	CD33	CD34	CD40	CD56	CD57	CD62L	CD66b	CD68	CD80	CD83	CD86	CD205	DC-Sign	HLA-DR	VEGR1	IL4-Rα	CD115	Reference
mRCC	ND	−	Int	ND	Int	Int	−	ND	−	−	−	ND	+	ND	ND	−	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	83
	ND	ND	ND	+	+	+	−	+	−	ND	ND	+	+	ND	ND	ND	ND	−	+	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	84
	ND	ND	ND	ND	+	ND	−	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	−	−	ND	ND	ND	ND	ND	ND	64
	ND	ND	ND	ND	ND	ND	−	+	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	85
	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	85
	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	86
	ND	−	ND	ND	ND	ND	−	ND	ND	−	ND	ND	+	ND	ND	−	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	87
Melanoma	ND	ND	+	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	+	+	ND	+	low	ND	+	ND	66
	ND	−	ND	ND	+	ND	+	ND	ND	−	−	ND	ND	ND	ND	−	−	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	69
	+	−	ND	ND	+	ND	−	int	ND	−	−	ND	ND	ND	ND	−	−	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	69
	ND	−	ND	ND	+	ND	−	−	ND	−	−	ND	ND	ND	ND	−	−	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	69
	ND	ND	ND	ND	ND	ND	−	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	88
Solid malignancies	ND	−	ND	ND	+	ND	−	ND	ND	−	ND	ND	+	ND	ND	ND	−	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	60
	high	ND	ND	ND	ND	ND	ND	ND	ND	− or +	ND	ND	+	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	89
HNSCC	ND	−	ND	−	ND	ND	−	−	ND	−	ND	ND	+	−	−	ND	−	ND	ND	ND	ND	ND	−	ND	ND	−	ND	ND	+	7
	ND	ND	ND	ND	+	ND	+	ND	ND	ND	ND	ND	+	+	ND	ND	ND	ND	ND	−	ND	ND	ND	ND	ND	low	ND	ND	ND	67
Breast cancer	ND	−	ND	−	ND	ND	−	−	ND	−	ND	ND	+	−	−	ND	−	ND	ND	ND	ND	ND	−	ND	ND	−	ND	ND	+	7
HCC	ND	ND	−	+	+	ND	+	−	−	−	ND	ND	+	−	ND	ND	ND	ND	ND	ND	−	−	+	ND	ND	low	ND	ND	ND	90
Pancreatic and esophago-gastric cancer	ND	−	ND	ND	+	ND	−	ND	−	−	−	ND	+	ND	ND	−	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	ND	ND	ND	57
Lung cancer	ND	−	ND	−	+	ND	ND	+	ND	ND	ND	ND	+	ND	ND	−	ND	ND	ND	−	−	ND	ND	−	ND	ND	ND	ND	ND	91
NSCLC	ND	ND	ND	ND	+	ND	−	+	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	92
	ND	−	ND	−	ND	ND	−	−	ND	−	ND	ND	+	−	−	ND	−	ND	ND	ND	ND	ND	−	ND	ND	−	ND	ND	+	7
Colon cancer	ND	ND	ND	ND	ND	ND	−	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	88
Prostate cancer	ND	ND	ND	ND	+	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	low	ND	ND	ND	68

Abbreviations: HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma ; int, intermediate; mRCC, metastatic renal cell carcinoma; ND, not determined; NSCLC, non-small cell lung cancer.

enough tumor material for research, almost all of the information on MDSCs in cancer patients has been obtained after research on peripheral blood samples. Whether this information truly correlates with the phenotype and function of MDSCs present within the tumor microenvironment, and, thus, under direct influence of factors secreted by these tumors, remains unclear. Significant controversy exists about the correlation between MDSCs and tumor stage and progression. A study conducted on peripheral blood samples from 106 cancer patients with newly diagnosed and histologically confirmed solid malignancies, revealed that there is a significantly higher percentage of circulating MDSCs in cancer patients relative to healthy volunteers.[7] In several other studies, including breast cancer, gastric cancer, melanoma, pancreatic and esophageal cancer, it has been shown that the frequencies of circulating MDSCs correlate with disease stage and metastasis and that the increase in the number of these MDSCs is an independent prognostic factor and could predict response to therapy.[57-59] Moreover, patients with stage IV solid tumors had the highest percentage of MDSCs. When they divided these stage IV patients into those with limited and those with extensive (defined as a diffuse involvement of one organ system or three or more distinct organ sites involved) metastatic tumor burden, the latter had both higher mean percentages and higher absolute numbers of circulating MDSCs. These data indicate that in different types of solid tumors, there is a correlation between MDSCs and both clinical cancer stage and tumor burden.[60] A study conducted in patients with stage IV melanoma performed at the University of Colorado Cancer Center showed that immunosuppressive cells, including T_{reg}, $CD14^+$ MDSCs and $CD14^-$ MDSCs, are specifically increased in metastatic melanoma patients and are found in association with each other. Moreover, a high frequency of $CD14^-$ MDSCs was shown to predict poorer survival of the patients and faster disease progression.[61] However, another study in patients with stage IV metastatic melanoma showed that the frequency of MDSCs was found to be rather low in melanoma patients and overlapped with the frequency detected in healthy donors.[62] In contrast, a study performed in Germany at the University Medical Center in Mainz showed that the accumulation of $CD11b^+CD33^+CD14^+HLA\text{-}DR^{low}$ MDSCs was an early event already detectable in stage I/II melanoma. Progression of disease (stage III) and high tumor burden in metastatic disease (stage IV) did not result in a further increase of MDSC frequencies nor in changes of phenotypic markers.[63] These contradictory results can be explained by different factors: differences in isolation methods used, different purification steps performed on the peripheral blood samples to isolate the MDSCs which may alter the natural frequencies of MDSCs, differences in cancer type, stage of disease and previous therapy of the patients. In order to compare data obtained in different laboratories it will be important to find a consensus about the markers used to define the phenotype of MDSCs and to perform studies on large groups of cancer patients.

Function

Although it has become clear in different murine tumor models that MDSCs derived from within the tumor microenvironment and those isolated from peripheral lymphoid organs have a distinct suppressive potential (see above), there are, to our knowledge, almost no studies comparing MDSCs from both sources head-to-head in cancer patients. The current understanding on the phenotype and especially the function of human MDSCs is almost completely derived from cells isolated from the peripheral blood of the patients and it is at this moment not clear yet whether this information is relevant for tumor-derived MDSCs as well. This could be partially explained by the difficulty to obtain tumor material from the clinic for research purposes, since this material is generally needed for diagnostic purposes. However, a few studies have made an attempt to investigate and compare the suppressive function of tumor-derived MDSCs and MDSCs isolated from the peripheral blood of cancer patients. A population of $CD14^-CD11b^+CD33^+$ cells, previously shown to bear characteristics of MDSCs,[64,65] were isolated from both the peripheral blood and tumor tissue obtained from three patients with head and neck cancer. In accordance with data obtained in tumor-bearing mice, MDSCs from within the tumor microenvironment produced significantly lower levels of ROS compared to blood-derived MDSCs while the iNOS levels were higher in tumor MDSCs than in blood MDSCs. Moreover, MDSCs derived from peripheral blood did not affect phytohemagglutinin (PHA)-induced T-cell proliferation, while MDSCs from the tumor microenvironment significantly suppressed T-cell proliferation.[25] This is in contrast with different studies showing suppressive activity of MDSCs derived from the peripheral blood of cancer patients.[66-68] However, in these studies a population of $CD14^+HLA\text{-}DR^{-/low}$ cells was investigated (in contrast to the $CD14^-CD11b^+CD33^+$ cell population) complicating direct comparisons of the suppressive function of these cells and again underlining the importance of consensus phenotypes to define MDSCs in cancer patients. In another study performed in patients with head and neck cancer, $CD14^+HLA\text{-}DR^{low}$ cells isolated from the tumor, the draining lymph node as well as the peripheral blood had the capacity to suppress antigen non-specific T-cell responses. However, also for this cell population it was clear that $CD14^+HLA\text{-}DR^{low}$ MDSCs from the tumor had a greater capacity to suppress autologous T cells compared to MDSCs derived from the peripheral blood.[67] Possible explanations for the discrepancy observed in the suppressive function of MDSCs obtained from the peripheral blood of patients with head and neck cancer include differences in phenotype of the cells under investigation, in stimuli used to induce T-cell proliferation and in handling of the peripheral blood samples. In the study performed by Corzo et al. the peripheral blood samples were treated in the same way as the tumor tissue (i.e. by enzymatic digestion), which could account for the loss of the suppressive function of MDSCs isolated from the peripheral blood.[25] In contrast to the two studies performed in patients with head and neck cancer, a study performed in metastatic melanoma patients showed that melanoma-infiltrating myeloid cells displayed an impaired suppressive capacity on antigen non-specific T-cell proliferation compared to cells with the same phenotype isolated from the peripheral blood of the same patient.[69] However, these melanoma-infiltrating $CD14^+$ cells expressed higher levels of HLA-DR compared to the cells

isolated from the peripheral blood, and perhaps this more differentiated phenotype can explain the lack of suppressive function of these cells in melanoma tumors compared to peripheral blood or other tumor types. Moreover, it remains unclear whether the inability of these cells to suppress non-antigen specific T-cell proliferation, as opposed to antigen-specific T-cell responses, reflects the real functional status of these cells in the tumor microenvironment. Another possible explanation is that the accumulation of MDSCs is less evident in an immunogenic tumor, such as melanoma, capable of responding to immunotherapy.[70-72] These data suggest that the contribution of MDSCs to the inhibition of T-cell proliferation in patients with metastatic melanoma may be less important than what was suggested by data obtained in murine tumor models. However, these are results from only a single study and more research will be needed in order to unravel the immunological relevance and the importance of MDSCs during tumor progression in cancer patients.

Trafficking of MDSCs

Infiltration of the tumor by inflammatory cells, including MDSCs, is an important factor in cancer progression. However, little is known about the mechanisms responsible for the mobilization and subsequent trafficking of MDSCs to the tumor site. Under normal conditions hematopoietic progenitors are predominantly found in the bone marrow, although low numbers can circulate in the blood. Mobilization is the first step in myeloid progenitor trafficking to inflammatory sites. The recruitment of MDSCs from the bone marrow to the peripheral blood can be mediated by Bv8 (Bombina variagata peptide 8, also known as prokineticin 1 and 2, a mitogen selective for endothelial cells) and endocrine-gland derived VEGF.[73,74] Also GM-CSF, secreted by the tumor cells, can mobilize MDSCs from the bone marrow. From the peripheral blood, MDSCs can be recruited to the tumor site by a number of chemokines, and the migration of particular MDSC subsets is strongly determined by the tumor histology and the spectrum of chemokines produced by these tumors.[75,76] The expression of several of these factors is increased by hypoxia, indicating that MDSCs might be preferentially recruited to sites of tumor hypoxia.[77] Myeloid cells also express integrins, such as $\alpha4\beta1$, the receptor for vascular cell adhesion molecule-1, which plays a role in the cellular trafficking to vascularized microenvironments.[78] MDSC accumulation also results from a prolonged survival and decreased apoptosis. It has been shown in a 4T1 model that the spleen, but not the bone marrow is the primary site for MDSC proliferation and serves as a reservoir from which MDSCs rapidly enter the bloodstream.[79]

Concluding remarks

Data obtained in different murine tumor models showed the importance of MDSCs in the suppression of T-cell responses and the promotion of tumor growth. Different studies showed that MDSCs present within the tumor microenvironment possess a stronger suppressive capacity compared to their non-infiltrating counterparts. Given the important role for MDSCs in the suppression of T-cell responses different studies focus on the specific targeting of MDSCs in order to alleviate the suppressive tumor microenvironment (reviewed in refs.[80,81]) and to achieve complete tumor regression. However, in cancer patients the role of MDSCs and the relative contribution of these cells to tumor progression is less clear. The major hurdle here remains the lack of a unified phenotype and standardized assays to determine the suppressive function of these cells. Without this, a comprehensive analysis and comparison of data obtained in different laboratories and clinical centers is very difficult and can possible lead to an under- or overestimation of the importance of MDSCs in the suppression of immune responses in cancer patients. One way to overcome this problem could be the development of a system that allows for the large-scale production of MDSCs ex vivo. Although different research groups are working on this topic, the methods published so far are still heterogeneous, complicated and characterized by a low yield. Moreover, the cells obtained by these methods may not resemble closely enough the cells that accumulate in the tumor microenvironment.[82] When the role of MDSCs in tumor progression and escape of immune control has become clear, strategies can be tested in order to reduce the number or inhibit the function of MDSCs, thereby creating a favorable tumor microenvironment for immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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