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Myeloid-Derived Suppressor Cells and anti-tumor T cells: a complex relationship

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Abstract

Myeloid-Derived Suppressor Cells (MDSC) are immature myeloid cells that are potent inhibitors of immune cell function and which accumulate under conditions of inflammation, especially cancer. MDSC are suggested to promote the growth of cancer by both enhancement of tumor angiogenesis and metastasis and also inhibition of antitumor immune responses. The presence of deficient and/or defective antitumor adaptive and innate immune responses, coincident with accumulation of MDSC in lymphoid organs and tumor parenchyma, supports the notion of a causal relationship. The potent ability of MDSC to inhibit several components and phases of immune response highlights the likelihood that targeting the inhibitory functions of MDSC may maximize the therapeutic potential of antitumor immunotherapy. In order to guide the rational development of immunotherapeutic strategies that incorporate inhibition of MDSC activity and enzymatic functions, thorough understanding of the role of MDSC in antitumor immune responses is required. In this manuscript we review the multifaceted inhibitory functions of MDSC and consider the role of MDSC-induced inhibition of antitumor T cell effector phase.

Keywords

myeloid-derived suppressor cells; tumor infiltrating t cells; Cancer

INTRODUCTION

As early as the 1890s, William Coley demonstrated the power of the immune system in combating tumor growth in that injection of erysipelas directly into tumor tissue produced remission in some sarcoma patients (Coley, 1894). That, and other, early work led to the prediction by Ehrlich that the immune system could provide protection against tumor growth. More recently, the accumulation of antigen-specific T cells within the tumor, draining lymph node, and the circulation show that the immune system can recognize protein products of genetic and epigenetic changes in transformed cells and that priming of the adaptive immune response occurs (Frey and Monu, 2006). Although variable in the magnitude of response, it has been conclusively shown that most cancers are immunogenic

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in situ. The nature of immune response to tumor growth is complex comprised of highly orchestrated and integrated innate and adaptive responses that reveal a delicate balance between positive and negative regulation (Ravetch and Lanier, 2000).

However, despite some exciting and successful examples, it is apparent that the immune system fails to eliminate tumor – most clinical trials using a wide variety of immune-based therapeutic approaches have failed (Ferris et al., 2010; Geldmacher et al., 2011; Rosenberg et al., 2004). Anti-tumor immune responses are either not sufficiently vigorous to eliminate the cancer or anti-tumor immunity is suppressed, thereby leading to tumor escape. The mechanisms of escape evolved by human tumors are varied, targeting multiple aspects of the immune response and creating a formidable hurdle for successful immune therapy.

Various mechanisms of tumor escape have been proposed (Gabrilovich and Haurwitz, 2008) including; restriction of priming or altered differentiation of anti-tumor T cells, which prevents either infiltration of antigen-specific T cells or activation of immune effector phase functions within the tumor microenvironment, and recruitment/activation of host suppressor cells such as FoxP3⁺ regulatory T cells and MDSC, which has been the focus of robust investigation in recent years.

Phenotypic Characterization of MDSC

MDSC, originally documented as “natural suppressor” cells in the bone marrow and spleen, are a heterogeneous and metabolically plastic cell population defined by their myeloid origin comprised of myeloid cell progenitors, and precursors of dendritic cells (DC), monocytes, macrophages, and granulocytes (Bronte, 2009). Plasticity in this usage refers to the ability of these cells to change both their expression of various biochemical mediators of suppression (e.g., iNOS, Arginase I (ARG1)) in response to environmental influences (e.g., local IL-4/13 or IFN- γ concentration) and also their differentiation state (e.g., becoming more/less neutrophil or myeloid in character). These cells are also defined by their incompletely differentiated (immature) state, and are characterized by the increased production of extracellular degradative enzymes, cytokines, and reactive oxygen and nitrogen species (Gabrilovich et al., 2001). In the non-inflamed state, cells having the phenotype of MDSC (‘Natural suppressor cells’) are long known to be present predominantly in the bone marrow (Strober, 1984), and participate in the normal process of myelopoiesis (controlled by a complex network of soluble factors including GM-CSF, G-CSF, M-CSF, SCF, FLT3 and IL-3).

Under various pathological conditions including cancer (in humans and mice), certain infectious diseases, sepsis, trauma, bone marrow transplantation and some autoimmune diseases, a variety of cytokines and soluble factors released induce rapid expansion of MDSC that accumulate in peripheral lymphoid organs, blood, and tumors, where they have been described to exert highly pleiotropic immune suppressive activities.

Tumor cells and host stromal cells in the tumor microenvironment secrete many factors (such as GM-CSF, VEGF, M-CSF, SCF, IL-6, IL-10, IL-1 β , prostaglandins (PGE₂), and the complement component C5a), and also express the enzyme cyclooxygenase that have been implicated in promoting recruitment and accumulation of immature myeloid cells (Bronte et al., 1999; Gabrilovich et al., 1998; Gabrilovich et al., 1996; Lin et al., 2002; Markiewski et al., 2008; Menetrier-Caux, C., et al., 1998, Park et al., 2004; Steinbrink et al., 2002). The unphysiologic levels and balance of factors is likely to influence the incomplete maturation of myeloid cells from bone marrow precursors resulting in accumulation of MDSC and also contributes to their immunosuppressive phenotype. These tumor-derived factors trigger many signaling pathways in MDSC mainly involving the signal transducer and activator of transcription (STAT) family of transcription factors (Gallina et al., 2006).

Specifically, STAT3 is constitutively activated in MDSC, and has been implicated as the principal transcription factor produced in response to stimulation by tumor-derived factors that prevents complete myeloid cell differentiation and thereby promotes MDSC expansion (Kortylewski et al., 2005; Nefedova et al., 2005; Nefedova et al., 2005). It is thought that STAT3 regulates myeloid progenitor cell survival and proliferation through the expression of B-cell lymphoma XL (BCL-XL), cyclin D1, MYC and survivin.

In addition, recent findings suggest that STAT3 also regulates MDSC expansion by inducing the expression of S100 calcium-binding protein A8 (S100A8) and S100A9 (pro-inflammatory proteins also found in the tumor microenvironment) (Cheng et al., 2008; Sinha et al., 2008), MDSC express receptors for S100A8 and S100A9 on their cell surface and also secrete S100A8/A9 (Turovskaya et al., 2008), implying an autocrine positive feedback loop that promotes MDSC recruitment and sustains MDSC.

In mice, MDSC are commonly identified as cells that co-express the cell surface markers CD11b and Gr-1 (Gabrilovich and Nagaraj, 2009). Since there are several subpopulations within Gr-1⁺CD11b⁺ cells, recently several groups have further subcategorized MDSC into “monocytic” MDSC (CD11b⁺Ly6G[−]Ly6C^{high}) and “granulocytic/ neutrophil-like” MDSC (CD11b⁺Ly6G⁺Ly6C^{low}) based on the expression of Ly6C and Ly6G (Movahedi et al., 2008; Youn et al., 2008; Zhou et al., 2010). In humans, MDSC are typically defined as CD11b⁺CD33⁺CD34⁺CD14[−]HLA-DR[−], which also vary in expression of other markers such as CD15 (human MDSC do not express a marker that is homologous to mouse Gr-1), and subsets of MDSC defined by their cell surface phenotype may possibly express either different biochemical mediators of suppression or different amounts of the mediators (Almand et al., 2001; Gabrilovich and Nagaraj, 2009; Norian et al., 2009; Ochoa et al., 2007; Schmielau and Finn, 2001).

Discrepancies exist in cell surface expression of certain activation/maturation markers, such as MHC II and costimulatory molecules, and of lineage markers (e.g., F4/80) between MDSC from different tumor-bearing mice and from individuals with different tumors. This heterogeneity supports the notion that MDSC include multiple subpopulations of myeloid-derived cells that are at various stages of maturity. Whether MDSC heterogeneity represents cells arrested in various but distinct stages of a linear differentiation program or separate committed lineages of cell subsets (but may be arrested in progress) is unclear. Whatever the lineage of a given population of MDSC, the apparent cellular heterogeneity (which may reflect distinct functional correlates in a given cell type) highlights the difficulty in identifying a particular cellular target for immunotherapy in neoplastic disease.

***In vivo* Manipulation of MDSC**

Schreiber and colleagues were among the first to show that depletion of granulocytes with anti-Gr-1 mAb could have therapeutic benefit in mouse tumor models *in vivo* (Pekarek et al., 1995). Other treatments that have been reported to reduce the levels of MDSC including: the chemotherapeutic drug gemcitabine (Sinha et al., 2007; Suzuki et al., 2005), or retinoic acid (Kusmartsev and Gabrilovich, 2003; Mirza et al., 2006), or the debulking of tumors (Sinha et al., 2005a, 2005b), as well as *in vivo* inactivation of genes that govern MDSC accumulation (Kortylewski et al., 2005; Nefedova et al., 2005; Sinha, P., et al., 2005a, 2005b; Terabe et al., 2003). These treatments can result in improved immune surveillance and immune cell activation and improved efficacy of cancer vaccines or other immunotherapies *in vivo*.

The systemic levels of MDSC appear to be related to tumor burden, as upon tumor resection, MDSC numbers decline dramatically (Salvadori et al., 2000) which is consistent with the notion that tumors produce myelopoeitic factors. MDSC can potentially impact on T cell

function in a variety of ways. As infiltration and accumulation of MDSC is a common feature of many tumor types, and MDSC produce a variety of pharmacologically active substances, collectively the abundant literature about MDSC suggests that these cells likely play a significant role in alteration of anti-tumor immune responses leading to diminished function.

MDSC Mechanisms of Suppression

MDSC, purified from tumor tissue or spleens of tumor-bearing mice, although likely containing several different cell types, induce defective T cell priming *in vitro*, which, if reflective of equivalent function *in vivo*, may restrict induction of tumoricidal immune responses. Similar to the phenotypic heterogeneity of MDSC, their targets and suppressive mechanisms are also heterogeneous. MDSC can contribute to tumor progression by facilitating neoangiogenesis (Kujawski et al., 2008; Shojaei and Ferrara, 2008; Shojaei et al., 2009; Shojaei et al., 2007; Yang et al., 2004) and by inhibiting innate and adaptive immunity via different mechanisms.

MDSCs have been implicated in altering anti-tumor immune responses indirectly by contributing to the suppressive network of the tumor microenvironment by facilitating tumor progression via regulation of angiogenesis and tumor cell motility. Briefly, MDSC are described as powerful promoters of angiogenesis by production of MMP and VEGF (Sica and Bronte, 2007; Yang et al., 2004), induced under the hypoxic conditions of the tumor microenvironment and regulated by the transcription factor hypoxia inducible factor (HIF) 1 α (Corzo et al., 2010).

The role of MDSC in perturbing innate immunity is less mechanistically defined, however, observations suggest that MDSC may inhibit innate immunity by suppressing NK cell-mediated lysis (Li, et al., 2009; Liu et al., 200; Nausch et al., 2008; Suzuki et al., 2005), and by polarizing tissue macrophage differentiation toward a type 2/‘alternatively activated’ phenotype (associated with tissue remodeling and pro-angiogenic activities) (Sinha et al., 2007; Sinha et al., 2005a), which enhance tumor progression. It has also been suggested that MDSC limit the availability of mature and functional DC, which bridge the gap between innate and adaptive immunity.

More robust investigation elucidated how MDSC may suppress T cell responses, and various potential mechanisms by which MDSC contribute to T cell nonresponsiveness in cancer is discussed here. MDSC potentially impact T cell function in a variety of ways, requiring cell-to-cell contact, can be antigen-specific or non-specific, and may depend on the specific MDSC subpopulation, the environment and the level of activation of target lymphocytes. This suggests a role for surface receptor interactions and/or short-lived soluble mediators.

Most MDSC-induced T cell suppression has been defined by *in vitro* assays using MDSC isolated from peripheral lymphoid organs (mostly spleen): inhibition of antigen-dependent cytokine secretion (Gabrilovich et al., 2001), induction of apoptosis in activated cells (Saio et al., 2001), secretion of a variety of factors having immunomodulatory properties (e.g., H₂O₂, TNF- α , NO, TGF- β), as well as production of enzymes that modulate amino acid metabolism [specifically tryptophan, arginine, and cysteine (Gabrilovich, 2004; Munn and Mellor, 2004; Serafini et al., 2004; Srivastava et al., 2010; Uyttenhove et al., 2003)], which have been associated with peripheral tolerance.

MDSC have also been shown to indirectly suppress T cell activation by inducing T regulatory cells (Tregs) (Huang et al., 2006), and recently demonstrated to impair T cell

homing to lymph nodes by regulating L-selectin levels (Hanson et al., 2009). Both these candidate mechanisms of immune suppression not discussed in this review.

MDSC Effects on Amino Acid Metabolism

L-arginine—The metabolism of L-arginine, influenced by nitric oxide synthase and arginase activity, has been extensively described in MDSC to affect the function of T lymphocytes (Bronte et al., 2003). In MDSC L-arginine is metabolized by ARG1, arginase II (ARG2), and the inducible form of nitric oxide synthase, (NOS2). Expression of ARG1 is regulated by Th2 cytokines, especially IL-4 and IL-13, and hydrolyzes L-arginine to urea and L-ornithine, the latter being the main substrate for the production of polyamines required for cell cycle progression and used by tumors to sustain their rapid proliferation (Chang et al., 2001; Wu and Morris, 1998). Increased ARG activity has been described in patients with colon, breast, lung, and prostate cancer (Cederbaum et al., 2004).

It has also been shown *in vitro* that L-arginine depletion induces loss of CD3- ζ chain in T cells, blocks T cell proliferation (being arrested in the G₀-G₁ phase of the cell cycle), and decreases cytokine production in T cells. Similar phenotypes have also been observed in T cells in cancer patients. For example, Ochoa and colleagues (Rodriguez et al., 2004) have shown that a subpopulation of tumor MDSC produces high levels of arginase, and not H₂O₂ or IDO, which inhibits proliferation of non-tumor infiltrating T cells *in vitro*. (In addition, tumor MDSC were shown to produce high levels of the cationic amino acid transporter, which is consistent with how MDSC might metabolize arginine at high levels in an arginine-deficient environment.)

Loss of cell-surface CD3 ϵ and CD3 ζ was observed coincident with the T cell proliferation defect, suggesting that arginine depletion caused the proliferation deficiency via down-regulation of key components of proximal TCR signaling machinery. [However, this notion is controversial since loss of CD3 ζ in TIL or systemic T cells has not been observed by others (Franco et al., 1995; Levey and Srivastava, 1995; Monu and Frey, 2007).]

A causal relation between MDSC production of arginase and antitumor T cell dysfunction was implied further by biochemical inhibition of arginase *in vivo* that resulted in diminished tumor growth rate. NOS2 is regulated by Th1 cytokines (IFN- γ , TNF- α) and oxidizes L-arginine to citrulline and nitric oxide (NO) (Bogdan, 2001). NO is a pleiotropic molecule that plays an important role in ischemia, inflammation, angiogenesis, immune response, and cell growth and differentiation (Bogdan, 2001). Increased production of NO within various human cancers may contribute to tumor development by favoring neoangiogenesis, tumor metastasis, and tumor-related immune suppression (Xu et al., 2002). It has been shown in several mouse tumor models that MDSC L-arginine metabolism via iNOS produces NO, which can also block T cell function by dampening IL-2 receptor signaling that is also associated with enhanced T cell apoptosis (Mazzoni et al., 2002; Saio et al., 2001).

Cysteine—Cysteine is another amino acid that mammalian cells require for protein synthesis and proliferation and is essential for T cell activation. It has been recently reported that MDSC also block T cell activation by depriving the environment of cysteine (Srivastava et al., 2010). Mammalian cells generate cysteine via different pathways. Cells expressing the plasma membrane cystine transporter x_c⁻, a heterodimer of 4F2 and xCT chains, imports disulfide-bonded cystine from the oxidizing extracellular environment and reduces it to cysteine in their intracellular reducing environment (Arner and Holmgren, 2000; Mansoor et al., 1992). Alternatively, cells that contain the enzyme cystathionase can convert intracellular methionine to cysteine (Gout et al., 2001; Ishii et al., 2004). T cells do not contain cystathionase or the xCT chain of the x_c⁻ transporter (Bannai, 1984; Eagle et al., 1966; Gmunder et al., 1990; Gmunder et al., 1991), so they cannot convert methionine to

cysteine and they cannot import cystine, making them dependent on other cells to produce cysteine, which is then imported through their ASC neutral amino acid plasma membrane transporter.

Under healthy conditions, antigen presenting cells, DC and macrophages, synthesize cysteine from methionine and convert imported extracellular cystine to cysteine, of which surplus cysteine is exported and imported by T cells during antigen presentation (Gmunder et al., 1990, Iwata et al., 1994). It was reported that while MDSC express the x_c^- plasma membrane transporter (permitting enhanced cystine uptake), MDSC do not express cystathionase (so are unable to convert methionine to cysteine), and do not express the ASC neutral amino acid transporter (so are unable to export cysteine). Hence, MDSC are fully dependent on importing cystine from the extracellular environment for conversion to cysteine. It is therefore suggested that contrary to DC and macrophages (which can import and export cysteine), MDSC that accumulate under neoplastic conditions, consume cystine in the extracellular environment (without exporting any cysteine), competing for and depleting the necessary amino acid for T cell activation and proliferation, resulting in inhibition of anti-tumor T cell responses (Srivastava et al., 2010).

Tryptophan—Tryptophan metabolism, originally associated with peripheral tolerance and maternal tolerance of the fetus (Grohmann et al., 2003; Gurtner et al., 2003; Hayashi et al., 2004; Kwidzinski et al., 2005; Munn et al., 1998), has also been linked to immunosuppression by MDSC cells in the tumor microenvironment. Tryptophan metabolism is increased in a tumor-conditioned microenvironment (Taylor, and Feng, 1991; Uyttenhove et al., 2003) and the activation of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) in DC has been implied to be involved in tumor immune evasion (Munn and Mellor, 2007). It was shown that human monocyte-derived macrophages and *in vitro*-derived DC expressing IDO inhibit T cell proliferation (Hwu et al., 2000; Munn et al., 1999; Munn et al., 2002). It is thought that myeloid derived cells that synthesize IDO might protect tumor from attack from specific tumor T cell by inducing tolerance during the priming phase or directly in the tumor microenvironment through tryptophan catabolism.

A caveat to considerations of mechanisms involving MDSC-mediated depletion of selected amino acids in the tumor microenvironment as causing blunting of local immune responses is that amino acid concentrations have not been directly measured in primary tumor. Thus, while data implicating such a mechanism are robust, they are ultimately inferential in nature lacking direct assessment. In the case of a candidate role for IDO it has furthermore been suggested that tryptophan metabolites are ultimately responsible for inhibitory effects on immune response, not depleted tryptophan levels (Lob et al., 2009; Lob, S., et al., 2009).

Reactive Oxygen and Nitrogen Species Production

MDSC in tumor bearing mice and patients with cancer are characterized by increased production of reactive oxygen species (ROS) induced by several tumor-derived factors. Supporting a role for ROS in MDSC-immune suppression, it has been shown *in vitro* that inhibition of ROS production by MDSC isolated from tumor-bearing mice and cancer patients completely abrogated the suppressive effects of MDSC. While it is commonly believed that ARG and iNOS are competitively regulated by Th1 and Th2 cytokines, ARG and iNOS can function synergistically in MDSC to inhibit antigen-specific T cell responses *in vitro* (Bronte et al., 2003, Wu and Morris, Jr., 1998).

When both enzymes are induced to sufficient levels, reactive nitrogen oxide species (such as peroxynitrites) are produced by NOS2 under conditions of limited L-arginine availability (Bronte et al., 2003). Peroxynitrites drive antigen-specific T cells to apoptosis by nitrotyrosylating key signaling proteins thus preventing tyrosine phosphorylation of these

proteins necessary for T cell activation (Brito et al., 1999). Recently, it has been shown that production of peroxynitrite by MDSC during direct contact with T cells resulted in nitration of the T-cell receptor (TCR) and CD8 molecules (Nagaraj et al., 2007). This resulted in conformational changes in the TCR-CD3 complex, altering its integrity and diminishing the physical interaction between CD8 and TCR, therein disrupting T cell signaling and rendering them unresponsive to antigen-specific stimulation (Nagaraj et al., 2010). Notably, non-specific TCR-CD3 complexes remained relatively intact and the same T cells are able to respond to non-specific stimuli (Nagaraj et al., 2010). Hence, the MDSC-induced defect via releasing peroxynitrites is only specific to cells bearing TCR involved in the interaction with the peptide presented by MDSC. These data provide biochemical support for the observation that tumor reactive T cells have diminished binding by epitope-specific tetramer (Nagaraj et al., 2007).

Further supporting a role for reactive nitrogen species in the inhibition of T cell response, it has been shown that in mouse tumor models, as well as during chronic helminth infection, T cell responsiveness to antigen can be restored by blocking the immunosuppressive activity of MDSC using peroxynitrite scavengers or the combination of ARG and iNOS inhibitors (Bronte et al., 2003; Brys et al., 2005; De Santo et al., 2005; Kusmartsev and Gabrilovich, 2005).

Do MDSC Inhibit the Antitumor T Cell Effector Phase?

Most of the findings discussed here describe MDSC-induced suppression of T cell response in the priming phase – little has been done to describe the suppressive activity, if any, of MDSC on the effector phase of cytotoxic T cells. Using a murine model of colon carcinoma (MCA38) our studies have focused on elucidating CD8⁺ tumor infiltrating lymphocyte (TIL) lytic dysfunction and the immunosuppressive mechanisms at play in the tumor microenvironment. Part of our study has included the examination of the role of MDSC in the effector phase inhibition of TIL. TIL have long been recognized as being deficient in cytokine release, proliferation, and lytic function.

Our laboratory has pursued a murine model of TIL dysfunction wherein we noted that the defective TIL lytic phenotype was transient, being regained upon purification and culture *in vitro* (lytic TIL) (Koneru et al., 2005). Freshly isolated TIL (nonlytic TIL) are memory/effector cells, whose proximal TCR-mediated signal transduction is blocked such that tyrosine kinase activity is weak and calcium flux is abrogated, deficiencies that undoubtedly underlie lytic dysfunction (Koneru et al., 2005). Inhibition of TCR signaling is manifested by the failure to activate ZAP70 and involves activation and recruitment to the immune synapse of the protein tyrosine phosphatase Shp-1, which is involved in inactivation of p56^{lck}, the most proximal tyrosine kinase in the TCR signaling cascade. As a consequence all downstream signaling is effectively blocked in TIL and effector phase functions are inhibited (Koneru et al., 2005; Monu and Frey, 2007).

L-arginine metabolism has been predominantly described as a mechanism utilized by MDSC to suppress proliferation of T lymphocytes in cancer patients and murine tumor models and it has also been shown that tumor cells can also suppress TIL effector functions via ARG1 and iNOS activity. Hence, we asked if TIL lytic dysfunction is mediated by iNOS and/or arginase. First, primary tumor cells, tumor cells depleted of MDSC, or purified MDSC were tested for the expression of iNOS after culture in the presence or absence of IL-4 (which induces arginase) or IFN- γ (which induces iNOS). Total primary tumor cells and purified MDSC express iNOS that is inducible by IFN- γ (Monu and Frey, 2007) (summarized in Table 1). Culture supernatants from primary tumor cells or MDSC were assayed for nitrite production *in vitro*, which showed that iNOS was active in MDSC even without IFN- γ treatment. Similarly, primary tumor cells were assayed for nitrite production and showed

strong arginase activity in MDSC ((Monu and Frey, 2007) summarized in Table 1). We also observed that there is no dramatic difference in nitrotyrosylated proteins between nonlytic and lytic TIL (data not shown), an observation that suggests a mechanism of T cell dysfunction distinct from that described above (Nagaraj et al., 2007).

To test the role of MDSC in the induction of TIL lytic dysfunction, co-culture of nonlytic or lytic TIL in contact with either purified MDSC or primary tumor cells depleted of MDSC was performed (Experimental procedure described in Fig. 1). Surprisingly, in spite of the evident iNOS and arginase activities expressed by MDSC, nonlytic TIL cultured with MDSC recover lytic function (Fig. 2; top panel); similarly lytic TIL are not induced to the nonlytic phenotype (Fig. 2; bottom panel). However, primary tumor cells effectively depleted of MDSC (and also the tumor cell line carried *in vitro* – (Monu and Frey, 2007)) both prevent the recovery of lytic function in freshly isolated nonlytic TIL and induce lytic dysfunction in lytic TIL (Fig. 2; top and bottom panel, respectively).

These data prompted the conclusion that tumor, not tumor MDSC (or regulatory T cells also present in the tumor), suppress TIL lytic activity in a contact-dependent manner. Further investigation identified Shp-1 playing an essential role in the observed defective TIL signaling. Our data show that Shp-1 activity is enhanced in nonlytic TIL, inhibition of p56^{lck} activity and lytic function is rapidly induced upon tumor contact, and inhibition of Shp-1 activity in lytic TIL prevents tumor-induced TIL lytic dysfunction (Monu and Frey, 2007).

Inhibition of TIL lytic function by tumor contact was interpreted to mean that MDSC do not inhibit the effector phase of CD8⁺ antitumor T cells in spite of robust metabolic activity in tumor MDSC. It is possible that our *in vitro* analysis of the consequences of TIL interaction with MDSC do not faithfully represent MDSC function *in situ* in that TIL have been manipulated by isolation and brief culture *in vitro*. However, the observation that re-exposure of lytic TIL to tumor cells *in vitro* induces the same functional and biochemical phenotypes of freshly isolated nonlytic TIL and tumor MDSC do not, argues that purified TIL maintain responsiveness to inhibitory environmental cues, and that MDSC do not provide that signal.

Although MDSC do not disrupt antigen-specific TIL lytic function, tumor MDSC may produce polyamines utilized by the tumor for rapid proliferation *in situ*. In addition, accumulation of MDSC in peripheral lymphoid organs could play a role in inhibition of anti-tumor immune response by inhibiting priming of anti-MCA38 T lymphocytes. This hypothesis is supported by studies of Gabrilovich and colleagues (Gabrilovich et al., 1997). The suppressive mechanisms for inhibition of the priming phase may not be applicable to inhibition of the effector cytotoxic T cells in tumor sites by MDSC.

CONCLUSION

The host immune system is one of the most important elements for protection from and attack against tumor development. MDSC have emerged as a heavily investigated and potentially important regulator of immune responses in neoplastic disease and several mechanisms have been described (using *in vitro* analysis and mouse tumor models) for MDSC induced suppression of anti-tumor immune responses. However, there are many questions yet to be answered in the field of MDSC biology and their potential *in vivo* impact on suppression of anti-tumor immunity and clinical relevance. It is still unclear what subset of MDSC may be responsible for T cell suppression and what the specific nature of MDSC-suppression is, i.e., antigen dependent or independent.

There are publications that support the notion of antigen-specificity in MDSC function and also non-specific functions. This issue is unable to be definitively resolved by review of the

literature but might be considered in light of the likelihood that antigen-activated immune cells express the same activation markers and activated signaling molecules as non-antigen-activated cells that are potential targets for MDSC inhibition.

More importantly, it is unclear if a dominant mechanism of MDSC-induced T cell suppression *in vivo* exists, as well as, if MDSC are suppressive to T cells in the periphery during the priming phase (overwhelmingly supported by *in vitro* findings) and/or in the tumor microenvironment during the effector phase. Although our findings which eliminate a role for MDSC in TIL lytic phase dysfunction (and identifies tumor cells as responsible for induction of TIL signaling and lytic defects) have been validated by others (Watanabe et al., 2008), this mechanism may be tumor- or model-specific. A comprehensive understanding of the mediators regulating MDSC recruitment, accumulation, differentiation, and suppressive activity under neoplastic conditions, will provide invaluable information for developing strategies of cancer immunotherapy and for drug design to overcome anti-tumor immune defects.

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Figure 1.

Schematic description of model. Primary tumor cells are isolated and CD8⁺ tumor infiltrating T cells ('nonlytic TIL') isolated by magnetic immunobeads. TIL function can be assessed immediately by cytotoxicity assay (shown at right) or TIL can be briefly cultured *in vitro* in ordinary culture medium lacking any exogenous cytokines (Radoja, S., et al., 2001) during which time lytic function is regained ('lytic TIL'). Total primary tumor cells, purified MDSC, or tumor cells after depletion of MDSC can be co-cultured with either lytic or nonlytic TIL and cytotoxicity assessed.

Figure 2.

Cytolysis assay of lytic and nonlytic TIL. TIL lytic function was assessed after co-culture with primary tumor cells, primary tumor cells depleted of MDSC or purified MDSC using cognate MCA38 tumor cells as target as described (Monu and Frey, 2007). There is no lytic function in freshly-isolated TIL and brief culture in the absence of tumor permits 'recovery' of antigen-specific lytic function as described (Koneru et al., 2005, Monu and Frey, 2007, Radoja et al., 2000, Radoja et al., 2001).

Table 1

Expression of reactive nitrogen species by primary tumor cells or purified MDSC.

	iNOS Expression (induced by IFN- γ)	Nitrite Secretion (induced by IFN- γ)	Urea Secretion (induced by IL-4)
Total Primary Tumor	++	++	++
Tumor depleted of MSC	+	+	+
Tumor MSC	+++	+++++	+++++

Primary tumor cells, primary tumor cells following depletion of MDSC, or MDSC purified by positive magnetic immunobeads were tested for urea and nitrite secretion by analysis of culture supernatants and cell extracts assayed for iNOS protein by immunoblotting as described (Monu and Frey, 2007).