The Power of Negative Thinking: Which Cells Limit Tumor Immunity?

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Running title: MDSC in Melanoma
Why human tumors grow infiltrated by specific anti-tumor T-cells has been a mystery attributed to negative factors released directly by the tumor or indirectly through immune intermediaries. The frequency and phenotype of myeloid derived suppressor cells (MDSC) in the peripheral blood of melanoma patients and healthy donors are surprisingly similar.

In this issue of Clinical Cancer Research, Gros and colleagues [1] have found that, unlike what has been reported in murine models, that MDSC are comparable in patients and normal individuals. Even more surprising, the study showed that the tumor infiltrating MDSCs are unable to suppress T-cell proliferation when compared with the peripheral derived MDSC. This stands in contrast to most of the studies performed in mice.

MDSC play an important role in regulating immune response and promote tumor progression in multiple murine models. MDSC is a heterogeneous immature myeloid cell population that is characterized by Gr-1 and CD11b positivity in the mouse and subdivided into monocytic (Mo) MDSC (CD11b^Ly-6G^Gr-1^high^) and granulocytic (G-) MDSC (CD11b^Ly-6G^Gr-1^low^)  Figure 1. There is no uniform marker for human MDSC but an increased frequency of Lin CD14^CD11b^CD33^CD15^HLA-DR^low^ myeloid cells and Lin CD14^-CD11b^-CD33^-CD15^ granulocytes are found to have immune suppressor function in the peripheral blood and have been similarly referred as Mo-MDSC and G-MDSC, respectively, in humans. MDSC have been identified in non-tumor settings in murine settings but have not been investigated in detail in most human disorders.[2] MDSC release factors including IL-1β, IL6, IL8, VEGF, TGFβ, MMP9 and reactive oxygen species (NO, ROS) which suppress T-cell proliferation, inhibit T-cell and NK-cell effector functions, attenuate T-cell migration and enhance angiogenesis, ultimately promoting tumor growth and progression.[3] While MDSC promote tumor growth, the tumor micro-environment can also enable generation of MDSC [4], thereby creating the tumor micro-environment - MDSC vicious cycle resulting in tumor growth. (Figure 1)

MDSC can be found in human melanoma patients’ peripheral blood or infiltrating the tumor.[4] While work has been done in characterizing the peripheral blood MDSC and its regulatory role, the functional role of human tumor infiltrating MDSC remained largely unexplored. In this issue of Clinical Cancer Research, the nominal suppressive function of melanoma infiltrating MDSC compared to its peripheral counterpart was carefully dissected. They classified the human myeloid cells into three categories: monocytic (CD14^-HLA-DR^+), granulocytic (CD14^-CD15^+), and eosinophilic (CD14^-CD15^INT^) cells. No statistically significant difference in the G-MDSC subset frequency was observed when comparing healthy individuals and melanoma patients’ blood. The frequency of Mo-MDSC is higher in melanoma patients when compared with healthy donors but this wasn’t statistically significant when enumerating viable cells. Previous studies have however shown that both the frequency of Mo-MDSC as well as G-MDSC in melanoma patients’ blood is higher when compared with healthy individuals [4, 5]. Previous studies have also found that Mo-MDSC accumulate in the periphery in stage II-III melanoma patients while they were not significantly different in advanced, stage IV melanoma patients. T-regulatory T-cells (T-reg) numbers, on the other hand, were significantly different and increased in stage IV patients. Although this paper nicely enumerated myeloid cells, it did not examine T-reg or T-effector cell function.
in melanoma patients’ periphery when compared with healthy donors. Perhaps mo-MDSCs play a more important role in the periphery in earlier stage of melanoma (II-III) while T-regs become more important in later stage of the disease (IV). Thus, targeting Mo-MDSC in early stage melanoma may be beneficial while targeting T-regs for later stages of disease may be more effective. Other tumor types would also be important to examine.

This group further characterized the melanoma infiltrating cells by flow cytometry following enzymatic digestion. Marked variability in the number of phenotypic tumor-infiltrating myeloid cells were identified. Higher CD14+ frequency and high HLA-DR+ cell numbers are found in the tumor-infiltrating myeloid cell population, implying a more active and differentiated state. Interestingly, they found a negative correlation between CD14+ infiltrating cells to T-reg number while the frequency of infiltrating G-MDSC was positively correlated with T-reg frequency. The mechanism by which infiltrating MDSC inhibit immunity may derive from the G-MDSC although they are present at lower frequency than Mo-MDSC. Furthermore, the ability to recover more granulocytic cells may have been limited by their viability.

Gros and colleagues also determined if the infiltrating MDSC isolated from melanoma tissue can inhibit T-cell proliferation in vitro. Surprisingly, when compared with the murine studies, neither tumor infiltrating G-MDSC nor Mo-MDSC can suppress anti-CD3+-driven gross T-cell proliferation while peripheral Mo-MDSC (HLA-DR or HLA-DR'), and CD14 CD15int (eosinophils) do. Whether the infiltrating MDSC suppress immunity via other mechanism, e.g. antigen specific mechanisms, is unknown. Induction of T-cell differentiation to a more Th2 phenotype than an anti-tumor Th1 phenotype following co-culture with peripheral derived MDSC or tumor infiltrating MDSC could still be an important mechanism not revealed by their assays. Although inhibiting T-cell proliferation is one of the key mechanisms by which MDSC inhibit immunity, induction of T-reg, impairment of effector function of T- and NK-cells, and enhancement of angiogenesis are also mechanisms by which MDSC could inhibit immunity and promote tumor growth. These areas remain unexplored and suitable areas for further study.

In summary, this study established that: 1) Mo-MDSC frequency but not the frequency of G-MDSC nor enumerated viable Mo-MDSC number is higher in melanoma patients when compared with healthy donors; 2) The frequency of tumor infiltrating G-MDSC but not Mo-MDSC have a positive correlation with the frequency of T-regs; and 3) Neither melanoma infiltrating G-MDSC nor Mo-MDSC can inhibit T-cell proliferation. Thus Mo-MDSC may be the responsible cell in the periphery while T-regs (induced by tumor-infiltrating G-MDSC) may be the critical cell within the tumor. Therefore, drugs such as all-trans-retinoic acid which promotes differentiation of myeloid progenitors [6] or sunitinib [7] which reduces MDSC accumulation may be more appropriate to target peripheral MDSC while small molecule drugs or antibodies that target Tregs at the tumor site could be a preferred strategy. Certainly the strategies designed to interrupt T-cell signaling with antibodies to CTLA-4 or PD1 suggest that a focus on the T-cell in patients might be more appropriate.

Since inhibition of T-cell proliferation seems not to be the primary mechanism for melanoma infiltrating MDSC to promote tumor growth, more detailed studies to determine how MDSCs recruit and function in tumor sites may provide us better insight to develop new strategies to target signals that induce their...
recruitment or activation. Indeed, it may be that damage associated molecular pattern molecules (DAMPs), released by the stressed or dying tumor could provoke tumor myeloid cells to up-regulate micro-RNAs as we have shown, capable of limiting immunity[8]. Alternatively enhanced resistance to immune effectors, possibly mediated by enhanced autophagy, might be directly regulated by cytokines or miRNAs released by tumors[9]. Melanomas are responsive to cytokine therapies such as IL-2 administration and it would have been of interest to determine whether tumors responding to IL-2 treatment would have a different set of myeloid or lymphoid cells in the tumor microenvironment, modifying the local and systemic autophagy observed[10]. Also, identifying molecules expressed by myeloid or lymphoid cells within the tumor microenvironment such as the Receptor for Advanced Glycation Endproducts (RAGE)[11] or the T cell Ig- and mucin-domain-containing molecule-3 (TIM-3)[12], which are inducible on myeloid and lymphoid cells might be informative to further dissect the powerful negative signals arising in the tumor microenvironment. Having murine tumor models which more closely resemble the tumor microenvironment in human tumors, perhaps with spontaneous tumors or slower growing tumors is also apparent as an important goal.
Reference


Figure Legend

**Figure 1. In the setting of human cancer, myeloid-derived suppressor cells (MDSC) increase in both the periphery and at the tumor site.** MDSC and tumor cells form a feedback loop which promotes tumor growth and progression. Tumors can release factors, including so-called Damage Associated Molecular Pattern Molecules or DAMPs, that favor MDSC generation in humans. MDSC is a heterogeneous population of myeloid cells which consist of two major subsets: monocytic (mo-MDSC) and granulocytic (MDSC), characterized by individual phenotypic markers in human and mouse. MDSC release factors that inhibit immunity by blocking T-cell and NK cell effector function and inhibiting T cell proliferation while promoting angiogenesis and T-regulatory cell growth. The ouroboros of tumor biology, promoting MDSC recruitment and activation, leads to even further tumor growth.
Subset
Phenotypic markers

Human (Lin⁻ CD14⁺ CD11b⁺ CD33⁺ CD15⁻ HLA-DRlow)
Mouse (CD11b⁺ Ly-6G⁻ Gr-1high)

Human (Lin⁻ CD14⁺ CD11b⁺ CD33⁺ CD15⁻ HLA-DRlow)
Mouse (CD11b⁺ Ly-6G⁻ Gr-1low)

Soluble factors

IL1β, IL6, IL8, VEGF, TGFβ, NO, ROS
NO, ROS, MMP9

Functional effect

Immunity
Block T-cell effector function, inhibit the release of TNF;
Impair T-cell proliferation via interaction with the TCR,
inhibit migration of T lymphocytes, increase T-regulatory
cells, suppress NK cell function

Angiogenesis
Promote angiogenesis, acquire endothelial cell properties
within the tumor micro-environment

DAMPs and Tumor Factors
S100A9, Serum Amyloid A-1, IL10, exosomes-miRNA (34c, 214), acidic pH, HMGB1, heat shock proteins

Promote tumor growth and progression

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