Published OnlineFirst August 21, 2012; DOI: 10.1158/1078-0432.CCR-12-2418

Clinical Cancer Research

CCR Translations
Commentary on Gros et al., p. 5212

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

Siuwah Tang and Michael T. Lotze

Why human tumors grow infiltrated by specific antitumor 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 in the peripheral blood of melanoma patients and healthy donors are surprisingly similar. *Clin Cancer Res; 18(19); 5157–9. ©2012 AACR.*

In this issue of *Clinical Cancer Research*, Gros and colleagues (1) have found that, unlike what has been reported in murine models, that myeloid-derived suppressor cells (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 MDSCs. This stands in contrast with most of the studies conducted in mice.

MDSCs 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 ($CD11b^+Ly\text{-}6G^-Gr\text{-}1^{high}$) and granulocytic (G-) MDSC ($CD11b^+Ly\text{-}6G^+Gr\text{-}1^{low}$), as shown in Fig. 1. There is no uniform marker for human MDSC, but an increased frequency of $Lin^-CD14^+CD11b^+CD33^+CD15^-HLA\text{-}DR^{low}$ 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 nontumor settings in murine settings but have not been investigated in detail in most human disorders (2). MDSC release factors including interleukin (IL)-1β, IL-6, IL-8, VEGF, TGF-β, matrix metalloproteinase 9 (MMP9) and reactive oxygen species (NO, ROS), which suppress T-cell proliferation, inhibit T-cell and NK-cell effecter functions, attenuate T-cell migration, and enhance angiogenesis, ultimately promoting tumor growth and progression(3). Although MDSCs promote tumor growth, the tumor microenvironment can also enable generation of MDSCs (4), thereby creating the tumor microenvironment—MDSC vicious cycle resulting in tumor growth (Fig. 1).

MDSCs can be found in human melanoma patients' peripheral blood or infiltrating the tumor (4). Although work has been done in characterizing the peripheral blood MDSC and its regulatory role, the functional role of human tumor infiltrating MDSCs remained largely unexplored. In this issue of *Clinical Cancer Research*, the nominal suppressive function of melanoma infiltrating MDSC compared with its peripheral counterpart was carefully dissected. The authors classified the human myeloid cells into 3 categories: monocytic ($CD14^+$ $HLA\text{-}DR^+$), 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 was not statistically significant when enumerating viable cells. However, previous studies have shown that both the frequency of Mo-MDSC as well as G-MDSC in melanoma patients' blood is higher when compared with levels in healthy individuals (4, 5). Previous studies have also found that Mo-MDSCs accumulate in the periphery in stage II–III melanoma patients, whereas they were not significantly different in advanced, stage IV melanoma patients. Regulatory T cell (Treg) numbers, on the other hand, were significantly different and increased in stage IV patients. Although this article nicely enumerates myeloid cells, it does not examine Treg 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 stages of melanoma (II–III), whereas Tregs become more important in later-stage disease (stage IV). Thus, targeting Mo-MDSC in early-stage melanoma may be beneficial, whereas targeting Tregs for later stages of disease may be more effective. Other tumor types would also be important to examine.

These authors further characterized the melanoma-infiltrating cells by flow cytometry following enzymatic digestion. Marked variability in the number of phenotypic tumor-infiltrating myeloid cells was identified. Higher $CD14^+$ frequency and high $HLA\text{-}DR^+$ cell numbers are

Authors' Affiliation: Departments of Surgery, Immunology, and Bioengineering, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

Corresponding Author: Michael T. Lotze, University of Pittsburgh Cancer Institute, 5117 Centre Avenue, Pittsburgh, PA 15213. Phone: 412-623-6790; Fax: 412-623-1212; E-mail: lotzemt@upmc.edu

doi: 10.1158/1078-0432.CCR-12-2418

Published OnlineFirst August 21, 2012; DOI: 10.1158/1078-0432.CCR-12-2418

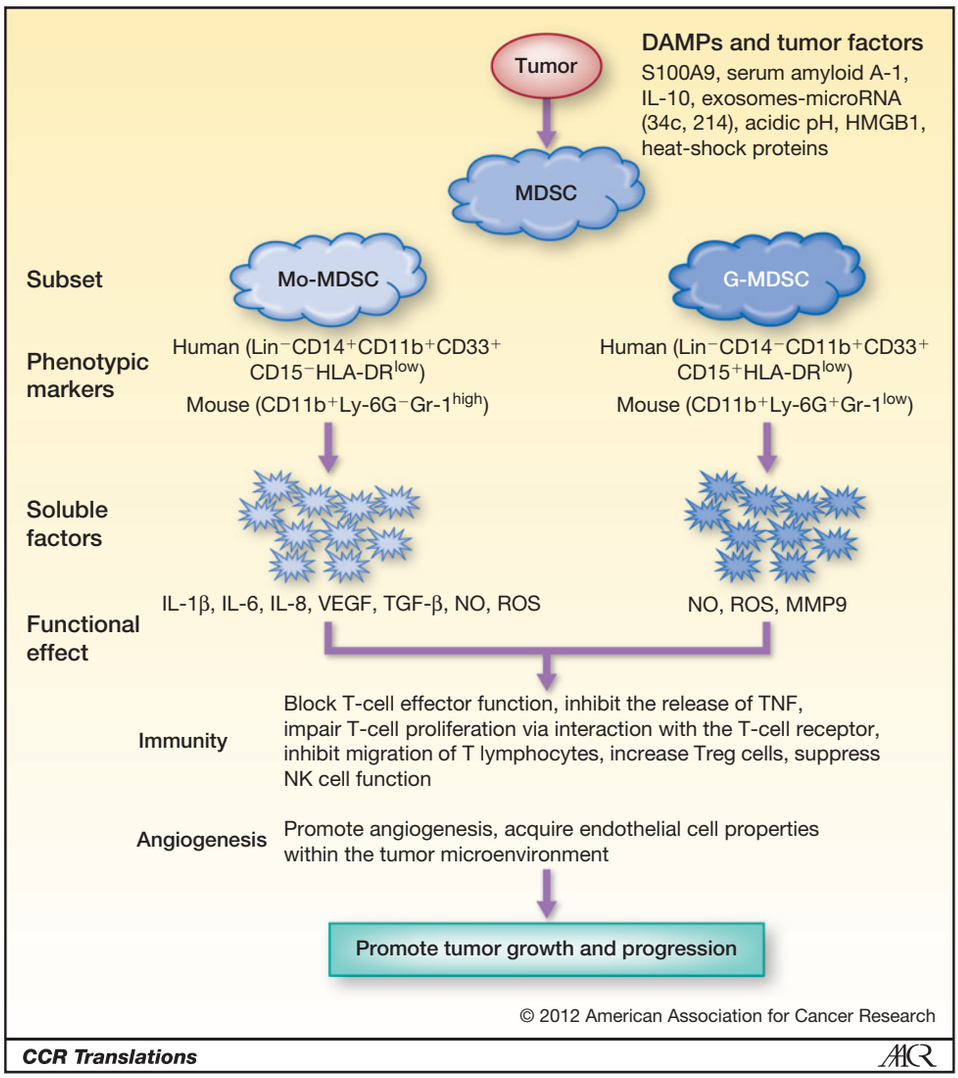

Figure 1. In the setting of human cancer, MDSCs increase in both the periphery and at the tumor site. MDSCs and tumor cells form a feedback loop that promotes tumor growth and progression. Tumors can release factors, including so-called damage associated molecular pattern molecules (DAMP) that favor MDSC generation in humans. MDSC is a heterogeneous population of myeloid cells that consist of 2 major subsets: monocytic (mo-MDSC) and granulocytic (G-MDSC), characterized by individual phenotypic markers in humans and mice. MDSC release factors inhibit immunity by blocking T-cell and NK cell effector function and inhibiting T-cell proliferation while promoting angiogenesis and Treg cell growth. The ouroboros of tumor biology, promoting MDSC recruitment and activation, leads to even further tumor growth.

found in the tumor-infiltrating myeloid cell population, implying a more active and differentiated state. Interestingly, the authors found a negative correlation between $CD14^+$ infiltrating cells and Treg numbers, whereas the frequency of infiltrating G-MDSC number was positively correlated with Treg frequency. The mechanism by which infiltrating MDSCs inhibit immunity may derive from the G-MDSCs, although they are present at lower frequency than Mo-MDSCs. Furthermore, the ability to recover more granulocytic cells may have been limited by the cells' viability.

Gros and colleagues also determined if the infiltrating MDSCs isolated from melanoma tissue can inhibit T-cell proliferation *in vitro*. Surprisingly, when compared with the murine studies, neither tumor-infiltrating G-MDSCs nor Mo-MDSCs can suppress anti-$CD3^+$-driven gross T-cell proliferation, whereas peripheral Mo-MDSCs ($HLA\text{-}DR^-$or $HLA\text{-}DR^+$) and $CD14^-CD15^{int}$ (eosinophils) do. Whether the infiltrating MDSCs suppress immunity via another mechanism, for example, antigen-specific mechanisms, is unknown. Induction of T-cell differentiation to a more Th2 phenotype than an antitumor Th1 phenotype following coculture with peripheral derived MDSCs or tumor-infiltrating MDSCs could still be an important mechanism not revealed by their assays. Although inhibiting T-cell proliferation is one of the key mechanisms by which MDSCs inhibit immunity, induction of Tregs, impairment of effector function of T cells and NK cells, and enhancement of angiogenesis are also mechanisms by which MDSCs could inhibit immunity and promote tumor growth. These areas remain unexplored and are suitable areas for further study.

In summary, this study established that (i) 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; (ii) the frequency of tumor-infiltrating G-MDSC but not Mo-MDSC has a positive correlation with the frequency of Tregs; and (iii) neither melanoma-infiltrating G-MDSC nor Mo-MDSC can inhibit T-cell proliferation. Thus, Mo-MDSC may be the

Published OnlineFirst August 21, 2012; DOI: 10.1158/1078-0432.CCR-12-2418

responsible cell in the periphery, whereas Tregs (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 MDSCs, whereas 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.

As inhibition of T-cell proliferation seems not to be the primary mechanism for melanoma-infiltrating MDSCs 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 (DAMP), released by the stressed or dying tumor could provoke tumor myeloid cells to upregulate microRNAs 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 microRNAs 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 end products (11) or the T-cell immunoglobulin- and mucin-domain–containing molecule-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 that more closely resemble the tumor microenvironment in human tumors, perhaps with spontaneous tumors or slower growing tumors, is also apparent as an important goal.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Tang, M.T. Lotze
Writing, review, and/or revision of the manuscript: S. Tang, M.T. Lotze
Other (developing proposal and working with S. Tang to prepare): M.T. Lotze

Received August 13, 2012; accepted August 16, 2012; published OnlineFirst August 21, 2012.

References

1. Gros A, Turcotte S, Wunderlich JR, Ahmadzadeh M, Dudley ME, Rosenberg SA. Myeloid cells obtained from the blood but not from the tumor can suppress T cell proliferation in patients with melanoma. Clin Cancer Res 2012;18:5215–23.
2. Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. Int Immunopharmacol 2011;11:802–7.
3. Filipazzi P, Huber V, Rivoltini L. Phenotype, function and clinical implications of myeloid-derived suppressor cells in cancer patients. Cancer Immunol Immunother 2011;61:255–63.
4. Filipazzi P, Valenti R, Huber V, Pilla L, Canese P, Iero M, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. J Clin Oncol 2007;25:2546–53.
5. Daud AI, Mirza N, Lenox B, Andrews S, Urbas P, Gao GX, et al. Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. J Clin Oncol 2008;26:3235–41.
6. 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 2008;66:9299–307.
7. 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.
8. Unlu S, Tang S, Wang EN, Martinez I, Tang D, Bianchi ME, et al. Damage associated molecular pattern molecule-induced microRNAs (DAMPmiRs) in human peripheral blood mononuclear cells. PLoS One 2012;7:e38899.
9. Yu Y, Cao L, Yang L, Kang R, Lotze M, Tang D. microRNA 30A promotes autophagy in response to cancer therapy. Autophagy 2012;8:853–5.
10. Lotze MT, Buchser W, Liang X. Blocking the interleukin 2 (IL2)-induced systemic autophagic syndrome promotes profound antitumor effects and limits toxicity. Autophagy 2012 Aug 1 [Epub ahead of print].
11. Kang R, Loux T, Tang D, Schapiro NE, Vernon P, Livesey KM, et al. The expression of the receptor for advanced glycation endproducts (RAGE) is permissive for early pancreatic neoplasia. Proc Natl Acad Sci 2012;109:7031–6.
12. Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol 2012;13:832–42.

AACR American Association for Cancer Research

Clinical Cancer Research

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

Siuwah Tang and Michael T. Lotze

Clin Cancer Res 2012;18:5157-5159. Published OnlineFirst August 21, 2012.