The Dawn of Myeloid-Derived Suppressor Cells: Identification of Arginase I as the Mechanism of Immune Suppression

Dmitry I. Gabrilovich

A study published in *Cancer Research* in 2004 by Rodriguez and colleagues identified the existence of arginase-producing myeloid cells in tumors distinct from macrophages. They demonstrated the role of arginase in negative regulation of T-cell function *in vivo*. This was one of the first reports implicating cells, which later were named myeloid-derived suppressor cells (MDSC), in T-cell suppression *in vivo* and linking this effect with arginase activity and expression. This work was important in advancing the field of MDSC research and helped to bring these cells to the forefront of cancer immunology.

See related article by Rodriguez et al., Cancer Res 2004;64:5839–49

Elimination of tumors by immunotherapy was always a very attractive, albeit quite elusive, goal of scientists and clinicians. The research effort to develop cancer immunotherapy was accelerated at the end of the last century with identification of various tumor-associated antigens. However, by the early 2000s, major challenges with this approach became evident. The immune-suppressive environment in tumors, as well as tumor-specific defects in T-cell responses, prevented the development of potent antitumor effects by therapies. Several different mechanisms were suggested to explain this phenomenon: accumulation of regulatory T cells, stromal barriers (mainly fibroblasts and mesenchymal stem cells) preventing infiltration of tumors by effector T cells, inhibition of T-cell function, and later, the phenomenon of T-cell exhaustion. Checkpoint molecules (such as CTLA4, PD-1) were proposed as possible mechanisms, which later changed the field of cancer therapy remarkably.

In the late 1990s, it became clear that one of the major factors limiting the function of T cells in the tumor microenvironment (TME) were tumor associated macrophages (TAM) and other not yet fully identified myeloid cells. Those cells were different from macrophages, expressed Gr-1 and CD11b markers, and had morphology of immature neutrophils and monocytes. Most importantly, they exhibited strong immune-suppressive activity toward T cells by inhibiting their proliferation and effector functions. This suppressive activity was observed not only in TME but in lymphoid organs and peripheral blood. Accumulation of these cells in tumor-bearing mice and patients with cancer was readily detectable by many investigators. By 2004, when Rodriguez and colleagues published their seminal article (1), limited information existed on the nature of the immune-suppressive activity of these cells. Moreover, many investigators involved in the study of neutrophils were highly skeptical about the entire possibility that immune-suppressive neutrophils exist and were biologically relevant in cancer. The research team headed by Dr. Augusto Ochoa investigated functionality of myeloid cells in cancer by studying arginase. They had previously demonstrated that depletion of essential amino acid L-Arginine reduced functionality of T cells. L-Arginine is metabolized by arginase I (Arg I), arginase II (Arg II), and the inducible nitric oxide synthase (iNOS). Arg I is located in cytoplasm, whereas Arg II in mitochondria. These enzymes hydrolyze L-Arginine and block signal transduction and functional activity of T cells. Rodriguez and colleagues were interested in the origin of arginase in TME and the mechanism of its effect on T cells. They sorted different populations of cells from mouse LL2 tumors and determined that arginase activity was associated with myeloid cells. They characterized those cells as mature $CD11b^{+}$ myeloid cells based on the expression of CD16/CD32 molecules and lack of the expression of the Gr-1 marker. These cells not only had high arginase activity but also increased expression of *Arg1*. This was associated with the decreased expression of CD3ζ and CD3ε chains on tumor-infiltrated T cells. The other noteworthy finding in this study was that tumor-associated myeloid cells had substantially lower production of reactive oxygen species (ROS) than nontumor-associated myeloid cells. Investigators linked downregulation of CD3ζ on T cells with their poor proliferation in response to antigen-specific stimulation. Higher arginase activity was also found in myeloid cells in patients with lung cancer. Finally, the authors demonstrated that inhibition of arginase with Nor-NOHA had antitumor activity *in vivo* (1).

This remarkable paper was published at the time when the existence of separate groups of myeloid cells with immune-suppressive activity was not accepted by most of the investigators. It demonstrated one mechanism, by which these cells could suppress T-cell function in cancer. In addition, this was one of the first reports demonstrating the possibility of therapeutic regulation of immune-suppressive TME to limit tumor progression. It helped to open a floodgate of studies that explored the biology of immune-suppressive microenvironment in cancer, which eventually led to the acceptance of the paradigm of immune-suppressive myeloid cells. Later, these cells were given the name myeloid-derived suppressor cells (MDSC), which is commonly used now (2). MDSCs are now considered a critical component

AstraZeneca, Gaithersburg, Maryland.

Corresponding Author: Dmitry I. Gabrilovich, AstraZeneca, 1 Medimmune Drive, Gaithersburg, MD 20878. E-mail: dmitry.gabrilovich@astrazeneca.com

Cancer Res 2021;81:3953–5

doi: 10.1158/0008-5472.CAN-21-1237

regulating immune response in cancer, chronic inflammation, infections, and autoimmune diseases. Accumulation of MDSCs is closely linked with negative clinical outcome in patients with cancer as well as with poor response to various immunotherapeutic strategies. Therapeutic targeting of these cells is one of the actively developing fields in cancer research.

As it is often the case with good papers, many of findings have different interpretations now. In 2004, MDSCs were considered as a single highly heterogenereous group of immature myeloid cells. It is now recognized that it consists of two major well-defined populations: monocytic (M-MDSC) and polymorphonuclear/granulocytic (PMN-MDSC). The view on immaturity of cells comprising MDSCs has also changed. It is now evident that although the majority of MDSCs are indeed immature neutrophils, there is a substantial proportion of fully mature MDSCs. Suppressive activity of MDSCs is not determined by whether these cells are mature or immature. The maturity of MDSCs apparently depends on the strengths of the signals produced by the tumor and speed of the production and mobilization of these cells from bone marrow. As a result, the morphology of MDSCs varies from one type of cancer to another (3).

MDSCs are defined as pathologically activated neutrophils and monocytes and are characterized by specific transcriptional and proteomic profiles, biochemical characteristics, and functional activity. High expression of Arg I and arginase activity is one of the major characteristics of MDSC (4). Although Rodriguez and colleagues suggested that cells they studied were different from immature myeloid cells, it is likely that they have described M-MDSCs. The cells in their study morphologically looked similar to monocytes. Low expression of Gr-1 found on those cells is typically associated with M-MDSCs, whereas high Gr-1 expression is typical for PMN-MDSCs. We now know that isolation of myeloid cells from tumors skewed resulting populations toward M-MDSCs due to the fact that PMN-MDSCs are more sensitive to isolation techniques.

Rodriguez and colleagues showed an important biological phenomenon that became a mainstream in the field: biochemical differences between myeloid cells in tumor tissues and in lymphoid organs. They showed much lower levels of ROS in tumor-associated myeloid cells as compared with cells outside tumors. This observation was confirmed by a number of studies. It could be due to the possibility that tumor-associated myeloid cells were likely enriched for M-MDSCs. However, there is now clear evidence that PMN-MDSCs and M-MDSCs in tumor sites have reduced ROS production as compared with spleen PMN-MDSC. In addition to ROS, tumor MDSCs have a much higher expression of iNOS and NO production than in peripheral lymphoid organs. As a result, tumor MDSCs are much more suppressive than their counterparts in the spleen or blood (5). Those observations were, of course, not known in 2004, but without this pioneer study, further progress would be much slower.

Subsequent studies from Ochoa's, Bronte's, and many other groups demonstrated that high *Arg1* expression is one of the hallmarks of MDSCs and allowed for a distinction between neutrophils and PMN-MDSCs (6). It is part of the MDSC signature in mice and humans (7).

Many studies demonstrated that inhibition of arginase *in vitro* abrogated suppressive activity of MDSCs. Moreover, some later studies showed that inhibition of arginase led to therapeutic response in mouse model of NSCLC (8). However, the issue is far from settled. The role of arginase in MDSC-mediated immune suppression was challenged in another study (9). Arginase inhibitor INCB001158 is currently in phase I/II clinical trial in patients with metastatic solid tumors in combination with chemotherapy (NCT03314935). Although clinical results were not impressive, the evaluation continues (10). One of the biggest challenges in targeting individual biochemical pathways of MDSCs is the high level of redundancy of many immune-suppressive mechanisms. Because MDSCs are pathologically activated myeloid cells, they utilize multiple mechanisms that may affect the function of immune cells. Besides arginase, these mechanisms include NO, peroxynitrite, superoxide and hydroxy peroxide, prostaglandin E2, TGFβ and other cytokines, adenosine, and many other molecules.

It appears that despite expression of multiple phenotypic markers, myeloid cells in cancer exist in two basic functional

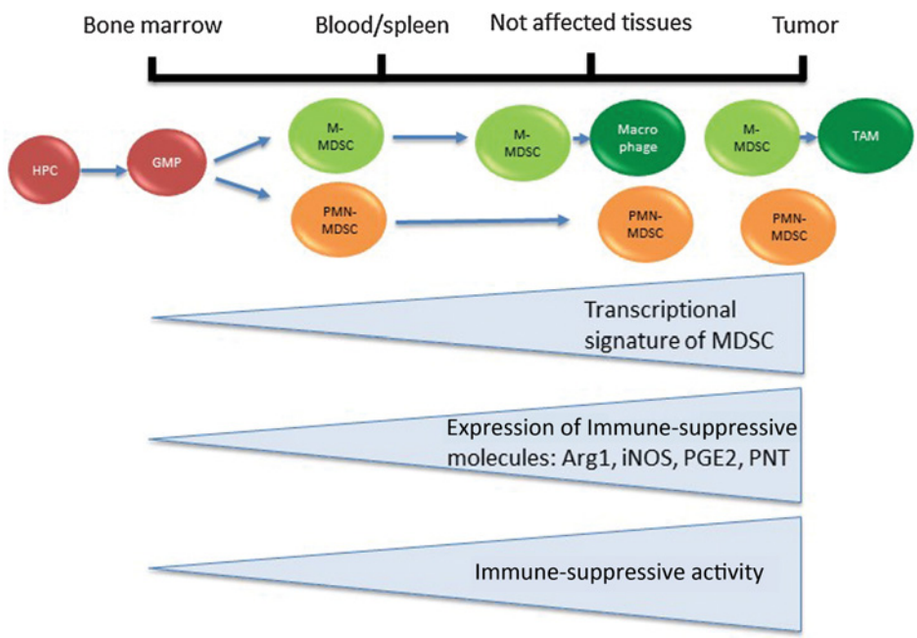

Figure 1.

The model of MDSC development. During differentiation from progenitors, under influence of tumor-derived factors, MDSCs acquire transcriptional changes that conditioned these cells. Whereas in bone marrow, these changes are relatively minor, they became more prominent in tissues, apparently as a result of an interaction with the tissue microenvironment. This manifests in a more potent suppressive activity of MDSCs. That effect becomes even more dramatic in TME where hypoxia, adenosine, and other factors dramatically augment activity of MDSCs.

states: tumor-promoting immune-suppressive cells (TAM, MDSCs) and classical macrophages, neutrophils and monocytes that lack immune-suppressive activity and sometimes can display antitumor activity. These functional states can be defined not only by the activity of these cells, but also by specific transcriptional profiles, which include among other markers *ARG1*. The nature of this functional dichotomy is not entirely clear, but one of the most appealing concepts is that in bone marrow, tumor-derived factors condition myeloid progenitors and precursors by causing acquisition of biochemical features normally not associated with typical (classical) neutrophils and monocytes. These characteristics are enhanced in cells after leaving the bone marrow, apparently as a result of an interaction with the tissue microenvironment. This manifests in a more potent suppressive activity of MDSC isolated from lung, liver, or other tissues than in the bone marrow or spleen. That effect becomes even more dramatic in TME where hypoxia, adenosine, and other factors dramatically augment activity of MDSCs (**Fig. 1**). Together, this suggests that targeting pathologically activated MDSCs by either eliminating or repolarizing these cells and sparing classically activated neutrophils and monocytes may provide an attractive therapeutic opportunity. The line of research that started in late 90s and was spearheaded by the study from Rodriguez and colleagues continues and promises new and exciting discoveries.

Authors' Disclosures

D.I. Gabrilovich reports other support from AstraZeneca outside the submitted work. No other disclosures were reported.

Received April 20, 2021; accepted April 20, 2021; published first August 2, 2021.

References

1. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 2004;64:5839–49.
2. Gabrilovich DI, Bronte V, Chen SH, Colombo MP, Ochoa A, Ostrand-Rosenberg S, et al. The terminology issue for myeloid-derived suppressor cells. Cancer Res 2007;67:425; author reply 6.
3. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. Nat Immunol 2018;19:108–19.
4. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol 2021 Feb 1:1–14. DOI: 10.1038/s41577-020-00490-y.
5. Veglia F, Hashimoto A, Dweep H, Sanseviero E, De Leo A, Tcyganov E, et al. Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. J Exp Med 2021;218:e20201803.
6. Zhou J, Nefedova Y, Lei A, Gabrilovich D. Neutrophils and PMN-MDSC: Their biological role and interaction with stromal cells. Semin Immunol 2018;35:19–28.
7. Ren W, Zhang X, Li W, Feng Q, Feng H, Tong Y, et al. Circulating and tumor-infiltrating arginase 1-expressing cells in gastric adenocarcinoma patients were mainly immature and monocytic myeloid-derived suppressor cells. Sci Rep 2020; 10:8056.
8. Miret JJ, Kirschmeier P, Koyama S, Zhu M, Li YY, Naito Y, et al. Suppression of myeloid cell arginase activity leads to therapeutic response in a NSCLC mouse model by activating anti-tumor immunity. J Immunother Cancer 2019;7:32.
9. Bian Z, Abdelaal AM, Shi L, Liang H, Xiong L, Kidder K, et al. Arginase-1 is neither constitutively expressed in nor required for myeloid-derived suppressor cell-mediated inhibition of T-cell proliferation. Eur J Immunol 2018; 48:1046–58.
10. Javle MM, Bridgewater JA, Gbolahan OB, Jungels C, Cho MT, Papadopoulos KP, et al. A phase I/II study of safety and efficacy of the arginase inhibitor INCB001158 plus chemotherapy in patients (Pts) with advanced biliary tract cancers. J Clin Oncol 2021;39 (no. 3_suppl), 311. DOI: 10.1200/JCO.2021.39.3_suppl.311.