

Tumor Escape Mechanism Governed by Myeloid-Derived Suppressor Cells

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Abstract

T-cell nonresponsiveness is a critical factor in immune escape and myeloid-derived suppressor cells play a major role in organizing this phenomenon. Recent findings indicate that myeloid-derived suppressor cells can induce antigen-specific CD8⁺ T-cell tolerance through a posttranslation mechanism which involves modification (nitration) of CD8 and the T-cell receptor itself on the T-cell surface. Elucidation of this mechanism of T-cell tolerance offers new opportunities for therapeutic corrections of immune escape in cancer. [Cancer Res 2008;68(8):2561–63]

Myeloid-Derived Suppressor Cells and Tumor Escape

Defective T-cell function represents one of the major mechanisms of tumor escape and one of the critical factors limiting the success of cancer vaccines. Tumor-induced T-cell abnormalities include antigen-specific nonresponsiveness (anergy/tolerance), deletion of T cells by apoptosis, and nonspecific suppression of T-cell function. In both animal models of cancer and in the clinical setting, unresponsiveness of T cells to tumor-specific antigens has been shown to be an early event in tumor progression. Studies have established that in tumor-bearing mice, a group of bone marrow-derived cells recently termed myeloid-derived suppressor cells (MDSC) are directly involved in the suppression of immune responses in cancer. These cells express both myeloid lineage differentiation antigen Gr-1 (Ly6G and Ly6C) and α_M integrin CD11b, and in control mice represent ~20% to 30% of normal bone marrow cells, 2% to 4% of all nucleated splenocytes, and are practically absent in lymph nodes. Inoculation with tumor cells or the development of spontaneous tumors results in a marked systemic expansion of these cells (1, 2). The number of MDSCs in spleens increase by 5- to 20-fold depending on the tumor model. These cells are also easily detected in lymph nodes or tumor sites. Similar expansion, albeit to a lesser degree, is observed in patients with cancer. In humans, MDSC are generally defined as CD14⁻CD11b⁺ cells, or more narrowly, as the cells that express the common myeloid marker CD33, but lack the expression of markers of mature myeloid and lymphoid cells (1, 2).

Numerous studies have established the potent immunosuppressive potential of MDSC. MDSCs may exert an immunosuppressive effect in both an antigen-specific and nonspecific manner depending on their localization and the specific characteristics of the tumor. Several different factors are implicated in MDSC-mediated immune suppression. They include arginase, reactive oxygen and nitrogen species, as well as inhibitory surface molecules. It seems that in the tumor site, the immunosuppressive activity of MDSC

is antigen-nonspecific and is primarily mediated by the production of nitric oxide (NO) in combination with a high arginase activity. NO could inhibit T cells via a variety of different mechanisms involving the blockade of phosphorylation and activation of Janus kinase 3, STAT5 transcription factor, inhibition of MHC class II gene expression, and induction of T-cell apoptosis. Arginase 1 activity causes the depletion of arginine and translational blockade of the ζ -chain of CD3. This prevents T cells from responding to various stimuli. High arginase activity in combination with increased NO production by the MDSC not only results in more pronounced T-cell apoptosis, but also leads to an increased production of reactive oxygen species (ROS) including peroxynitrites (ONOO⁻) and hydrogen peroxide (H₂O₂) by the MDSC (3). In this environment, T cells are suppressed irrespective of the nature of the antigens these cells could encounter. Profound immune suppression of tumor-infiltrating lymphocytes is a well-documented fact (4).

In contrast, in the peripheral lymphoid organs, MDSC have very little NO production and moderate levels of arginase but instead produce a large amount of ROS, primarily hydroxyl peroxide and peroxynitrite. Because ROS are very short-lived substances, MDSC are able to suppress T cells only during direct cell-to-cell contact. The most effective means to secure such contact is the antigen-specific interaction between MDSC and T cells. Because MDSC are able to take up, process, and present antigens, this may provide a mechanistic foundation for antigen-specific T-cell suppression in cancer (5, 6). Importantly, this antigen-specific nature of immune suppression may address an apparent paradox: why a number of studies have reported a lack of profound systemic immune suppression in tumor-bearing mice despite the presence of a very high number of immunosuppressive MDSC. Similarly, cancer patients usually show only moderate levels of systemic immune suppression unless they are treated with a high dose of chemotherapy or at the terminal stages of the disease.

Mechanism of MDSC-Inducible CD8⁺ T-Cell Tolerance

Although previous studies have implicated MDSC in tumor-associated CD8⁺ T-cell tolerance (5, 6), the mechanism of this phenomenon remains unclear. To address the potential role of MDSC in CD8⁺ T-cell tolerance in cancer, we used an experimental model which combined adoptive transfer of different transgenic CD8⁺ T cells and tumor-derived MDSC into naïve congenic recipients (5). CD8⁺ T cells from MDSC-treated mice were not able to produce IFN- γ and interleukin 2 in response to specific peptides and did not kill peptide-loaded target cells (5). At the same time, these CD8⁺ T cells responded well to stimulation with anti-CD3 antibody. MDSC induced a marked decrease in the binding of specific peptide-MHC (pMHC) complex to CD8⁺ T cells (7). Although this effect has not been previously described in cancer, it is known that the changes in TCR-binding avidity can play an important role in regulating antigen sensitivity (8). Tolerization of H-Y TCR transgenic mice by repeated pMHC administration

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resulted in a substantial decrease in MHC tetramer binding (9). Among the proposed mechanisms which could induce changes in TCR are the down-regulation of CD8 expression, changes in cellular localization of TCR, and glycosylation of TCR. However, in our experiments, MDSC did not induce the down-regulation of CD8 or TCR. MDSC defective in ROS production (gp91^{-/-} mice) were not able to induce T-cell tolerance and the use of a peroxynitrite scavenger completely eliminated MDSC-induced T-cell tolerance, suggesting that ROS, and peroxynitrite in particular, could be responsible for MDSC-mediated CD8⁺ T-cell tolerance (7).

Increased production of ROS has emerged as one of the major characteristics of MDSC. Previous studies have shown that oxidative stress, caused by MDSC derived from tumor-bearing mice, inhibited ζ -chain expression in T cells and antigen-induced cell proliferation (10). Because ROS production by MDSC can be blocked by arginase inhibitors, it seems that arginase I activity plays an important role in ROS accumulation in these cells (11). Schmielau and Finn observed that the immunosuppressive effects of myeloid cells with a granulocyte phenotype copurified with low-density peripheral blood mononuclear cells was abrogated by the addition of catalase, further implicating ROS in MDSC-mediated immune suppression (12). Interaction of MDSC with antigen-specific T cells in the presence of specific, but not control, antigens resulted in a significant increase of

ROS production. This increase was independent of IFN- γ production by T cells, and was mediated by integrins CD11b, CD18, and CD29 (11). This data was consistent with reports implicating adhesion molecules, and integrins in particular, in ROS production by macrophages and fibroblasts (13).

Peroxynitrite is responsible for most of the adverse effects linked to ROS. Peroxynitrite is a product of the reaction of NO and superoxide. The combination occurs at a diffusion-limited rate with NO outcompeting superoxide dismutase. As a result, peroxynitrite is present at the sites characterized by the accumulation of MDSC, inflammatory cells, or ongoing immune reactions. Peroxynitrite induces the nitration of several amino acids: cysteine, methionine, tyroptophane, and most prominently tyrosine.

We hypothesized that nitration of TCR and/or CD8 could alter the binding of the TCR/CD8 complex with pMHC, which is necessary for T-cell stimulation. Molecular modeling revealed a number of tyrosine residues in TCR and CD8 molecules that could be susceptible to nitration. Structural analysis showed that nitration of these tyrosine residues would result in decreased flexibility and increased rigidity of TCR domains that might significantly alter the epitope-specific interactions between TCR and pMHC, as observed in our study (7).

In our direct experiments, MDSC caused an increased level of nitrotyrosine on the surface of antigen-specific CD8⁺ T cells both

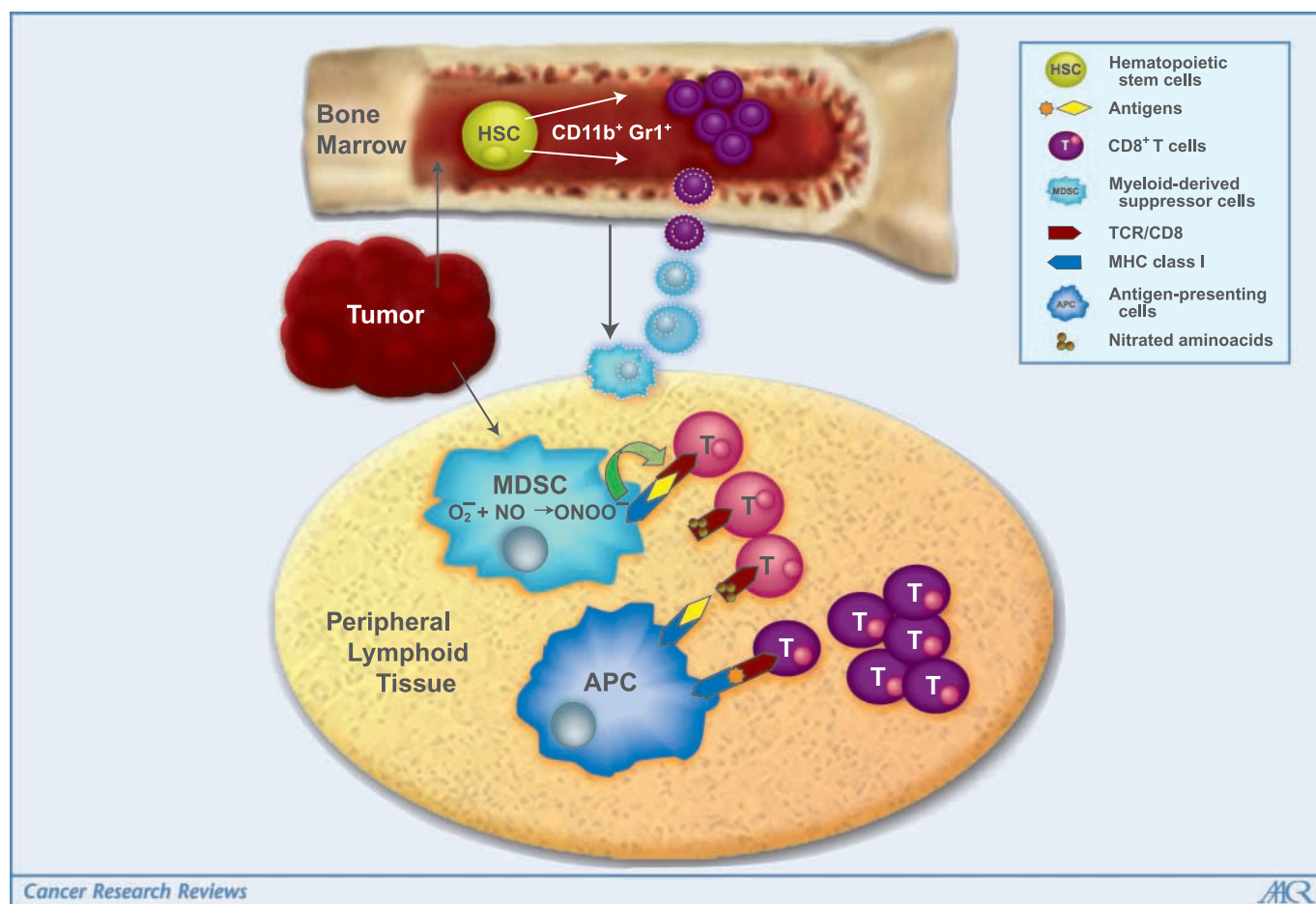


Figure 1. A model of T-cell tolerance in cancer induced by MDSC. Tumor-derived factors induce the production of MDSC from hematopoietic stem cells (HSC) in the bone marrow. MDSC migrate to the tumor site and peripheral lymphoid organs. In the peripheral lymphoid organs, MDSC retain high levels of arginase I activity and produce ROS including peroxynitrite. MDSC present antigen to antigen-specific T cells and during direct cell-cell contact induce nitration of TCR and CD8 molecules on the T-cell surface. This results in conformational changes in these molecules and loss of their ability to bind to pMHC. Ultimately, CD8⁺ T cells are rendered nonresponsive to antigen stimulation.

in vitro and *in vivo* (7). Immunoprecipitation showed that both TCR and CD8 molecules had increased amounts of nitrotyrosine. The blockage of nitrotyrosine generation using the peroxynitrite scavenger uric acid reversed the MDSC-inducible antigen-specific CD8⁺ T-cell suppression *in vitro* and *in vivo*, directly confirming the important role of peroxynitrite in MDSC-mediated T-cell non-responsiveness. T-cell dysfunction is one of the major factors limiting the effect of cancer vaccine. We then asked whether a block of peroxynitrite might improve the effect of cancer immunotherapy. To test this hypothesis, we treated tumor-bearing mice with uric acid in combination with a dendritic cell-based p53 vaccine. Such treatment substantially improved the antitumor effect of the vaccine (7).

A Model of MDSC-Inducible T-Cell Tolerance

These studies suggest a potential model of MDSC-inducible T-cell tolerance (Fig. 1). MDSC are generated in response to tumor-derived cytokines. These cells produce high levels of ROS, probably as a result of the effect of the same tumor-derived factors. MDSC are able to pick up soluble antigen, process, and present them on their surface. During the presentation of antigen, MDSC closely interacts with antigen-specific T cells. This provides an environment in which peroxynitrite abundantly produced by MDSC could affect molecules on the surface of T cells. We propose that peroxynitrite may modulate an immune response leading to anergy via modification of different amino acids in molecules involved in the recognition of peptide MHC complexes with TCR, CD8, and possibly CD4. Antigen-specific interactions provide much more robust and stable synapses than antigen nonspecific interactions (14). This may explain the antigen-specific nature of MDSC-mediated T-cell tolerance in the periphery. Modification of surface molecules, nevertheless, does not lead to T cell death. Moreover, T cells are capable of responding to nonspecific activation. This may help to explain the absence of profound systemic immune suppression in tumor-bearing mice despite the presence of a large number of MDSC.

This model is consistent with previously described immunosuppressive effects of peroxynitrite in cancer. Bronte and colleagues

reported that human prostatic adenocarcinomas are infiltrated by terminally differentiated CTLs. These lymphocytes, however, were in an unresponsive state. This group further showed the presence of high levels of nitrotyrosine in prostatic tumor-infiltrating lymphocytes, suggesting a local production of peroxynitrites. Restoration of tumor-infiltrating lymphocyte responsiveness to tumor could be achieved by simultaneous inhibition of inducible NO synthase and arginase activity (15). Recent studies have shown an important role of ROS in T-cell tolerance in an experimental model of arthritis. Low levels of ROS production, due to a mutation in the *Ncf1* gene coding for p47^{phox} subunit of the NADPH oxidase complex, was shown to break tolerance to collagen, resulting in enhanced T-cell autoreactivity, high titers of anticollagen antibody, and the development of severe arthritis (16). Peroxynitrites produced during inflammatory conditions were directly implicated in the promotion of tumor progression via modification and inactivation of different proteins. An association of high peroxynitrite levels with tumor progression was documented for pancreatic cancer, malignant gliomas, head and neck cancer, breast cancer, melanoma, and mesothelioma (17–21).

The concept of MDSC-inducible T-cell tolerance caused by posttranslational modification of surface molecules is far from being proved and many questions require further clarification. The antigen-specific versus nonspecific nature of peripheral T-cell suppression is still a matter of debate and probably very much depends on the presence of an inflammatory microenvironment in peripheral lymphoid organs. Additionally, the role of MDSC in CD4⁺ T-cell tolerance is not yet clarified. However, despite the fact that these issues are not yet resolved, it seems that blockade of peroxynitrite generation or the use of scavengers could represent an attractive opportunity to decrease tumor-associated T-cell tolerance and enhance the efficacy of cancer immunotherapy.

Acknowledgments

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