Chapter 13
Arginine Metabolism, a Major Pathway for the Suppressive Function of Myeloid-Derived Suppressor Cells

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Abstract Various mutations in cancer create a microenvironment surrounding the tumor, characterized by the presence of a chronic inflammatory infiltrate which facilitates the growth of the tumor cells, enhances angiogenesis and more importantly, inhibits any protective immune response. One of the most prominent inflammatory cells are the so-called myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature myeloid cells that are potent inhibitors of T cell, NK cell, and dendritic cell functions. Recent findings in tumor-bearing mice and patients with cancer indicated that the increased metabolism of the nonessential amino acid L-Arginine by MDSC-producing Arginase I inhibits T-cell-lymphocyte responses. Here, we discuss some of the most recent concepts of how MDSC expressing Arginase I may regulate T-cell function in cancer and suggest possible therapeutic interventions to overcome this inhibitory effect. In addition, we discuss how metabolic limitation of L-Arginine can be used as a novel therapy to downmodulate T-cell responses in several diseases. Altogether, this chapter emphasizes the importance of the metabolism of the amino acid L-Arginine as a regulator of inflammation-linked diseases and also suggests the potential use of this pathway as a therapy to control unbalanced T-cell responses in autoimmunity and transplantations.

Keywords Arginine · Myeloid-derived suppressor cells · Immune response · L-Arg starvation · Arginase I expression · tumors-infiltrating MDSCs · Hematopoietic progenitors · Molecular mechanisms · MDSCs in vivo

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1 Introduction

Current concepts in cancer development and progression have convincingly demonstrated that malignant tumors create a chronic inflammatory microenvironment that promotes their growth and invasive properties. Until recently, the presence of this inflammatory response was poorly understood. The bands of inflammatory cells and fibrotic tissues seen surrounding many solid tumors were thought to be the remains of a failed attempt by the immune system to control the growth of the malignant cells. Similarly, the high numbers of granulocytes found in the peripheral blood of some cancer patients without an active infection were classified as leukemoid reactions and were primarily considered to be a nonspecific effect of the continued tumor growth. Research during the past two decades has instead shown that these inflammatory cells are induced by tumors and play an important role in supporting carcinogenesis and their growth, invasion, and metastatic spread. The heterogeneous population of cells that make up this chronic inflammatory microenvironment is composed primarily of $CD11b^{+}$ myeloid cells that are highly suppressive of antitumor T-cell responses. However, they also promote angiogenesis, induce regulatory T cells, and even protect tumor cells from the effects of chemotherapy and radiation therapy. The molecular mechanisms used by these cells to suppress T-cell function include depletion of amino acids arginine, tryptophan, and cysteine, the production of reactive nitrogen species such as nitric oxide (NO) and peroxynitrites (NOO^{-}), and the production of reactive oxygen species (ROS). Here, we discuss the most recent data on how myeloid-derived suppressor cells (MDSCs) metabolizing L-arginine (L-Arg) may regulate the production of reactive nitrogen species and ROS and suppress T-cell function in cancer and other diseases.

2 Alterations of the Immune Response in Cancer

A dysfunctional immune response in cancer patients manifested by the loss of delayed-type hypersensitivity was demonstrated several decades ago, but the underlying mechanisms were unknown [62], [63], [110], [109]. Initial hypotheses included the presence of "blocking antibodies," the production of suppressor factors by tumor cells, and the generation of suppressor macrophages [31], [32], [107]. Murine models also showed that tumor growth was associated with a progressive decrease in T-cell function that could be reestablished through the use of prostaglandin inhibitors or low-dose chemotherapy [28], [29], [56]. These concepts were incorporated early into the immunotherapy trials where low-dose cyclophosphamide or local radiation was used as a preconditioning regimen preceding adoptive cellular therapy. However, early immunotherapy trials in human patients failed to reproduce the therapeutic successes seen in murine models (with 3–5-day-old tumors) (reviewed in [26]). In fact, several vaccine trials demonstrated that tumors were able to progress even in the presence of a strong T-cell response [102].

In the early 1990s, the identification of several discrete but specific alterations in T cells from mice or patients with cancer, such as a decreased expression of the T-cell receptor ζ chain (CD3ζ), a diminished level of the tyrosine kinases $p56^{lck}$ and $p59^{fyn}$, and the inability to upregulate Jak-3 and to translocate nuclear factor kappa B (NF-κB)–p65 [19], [45], [55], [62], [64], [114], [115], provided the first molecular mechanisms to explain the decreased T-cell response in cancer. Almost simultaneously, investigators developed cellular and molecular models that provided important insights into the multiple mechanisms by which cancer and chronic inflammatory diseases could selectively inhibit T-cell responses [41], [81], [101]. These models facilitated the discovery of immunoregulatory mechanisms such as the expression of checkpoint molecules on T cells and antigen-presenting cells (APCs) [18], [43], [48–50], the development of regulatory T cells [60], [61], and the accumulation of tumor-induced MDSCs [7], [20], [80], [93].

Using cocultures of activated murine peritoneal macrophages and T cells, Otsuji et al. [77] and Kono et al. [45], [46] first demonstrated that activated peritoneal macrophages cocultured with T cells induced the loss of the CD3ζ chain of the T-cell receptor and suppressed T-cell responses in vitro. This effect was blocked by the use of oxygen radical scavengers, suggesting that it was, in part, mediated by ROS [14]. Soon after, Schmielau et al. described the presence of an increased number of activated neutrophils in the peripheral blood of patients with advanced pancreatic and breast cancer who also showed a diminished expression of the CD3ζ chain [91]. Changes in the expression of the CD3ζ chain were also found in patients with metastatic renal cell carcinoma [117] where increased granulocyte counts were found to be associated with a poor outcome [82]. In addition, Zea et al. and Baniyash et al. [6], [115], [118] demonstrated that the changes in T cells were also found in infectious diseases such as tuberculosis and leprosy, suggesting that the chronic inflammatory microenvironment rather than the tumor cells were responsible for the induction of T-cell dysfunction.

Which tumor factors lead to the activation and/or accumulation of MDSCs? Gabrilovich et al. [113] and Bronte et al. [17] demonstrated that the vascular endothelial growth factor (VEGF), granulocytic colony-stimulating factor (G-CSF), and granulocytic–monocytic colony-stimulating factor (GM-CSF) produced by tumor cells arrested the differentiation of myeloid cells, resulting in the accumulation of immature myeloid cells (iMCs) that, in turn, suppressed T-cell function. These suppressive myeloid cells were found to be increased in patients with head and neck, breast, and lung cancer [2], [3] and were initially thought to block T-cell responses by producing interleukin-10 (IL-10), transforming growth factor beta (TGF-β), and prostaglandin E_2 (PGE_2). However, it soon became apparent that MDSCs had additional and more potent inhibitory mechanisms that had not previously been described.

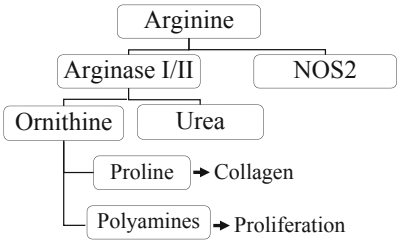

Fig. 13.1 L-Arginine metabolism in myeloid cells. L-Arginine is metabolized in myeloid cells through arginase I and II and nitric oxide synthase 2 (NOS2). L-Arginine hydrolysis through arginase I and II results in the production of urea and ornithine, the later being a major precursor for the synthesis of polyamines and collagen.

3 Metabolism of L-Arg by Myeloid Cells

In addition to the production of immunosuppressive cytokines, MDSCs were found to rapidly deplete the amino acid L-Arg from the microenvironment. L-Arg is the substrate for at least four enzymes that exist as multiple isoforms in MDSCs: nitric oxide synthases (NOS1, NOS2, and NOS3), arginases (arginase I and II), arginine glycine amidinotransferase (AGAT), and L-Arg decarboxylase (ADC) [66]. The normal serum concentration of L-Arg is maintained through a combination of dietary intake and de novo synthesis. Dietary L-Arg is taken up by intestinal epithelial cells and traverses the plasma membrane via the y+ system of cationic amino acid transporters (CATs) [13]. De novo synthesis of L-Arg occurs primarily in the kidney as a result of recycling of citrulline produced in the intestine [65]. Once L-Arg is transported into the cytoplasm, its metabolism depends on the type of cell. In myeloid cells, L-Arg is primarily metabolized by the inducible NOS (iNOS) or by arginase I or II (Figs. 13.1 and 13.2). iNOS metabolizes L-Arg to produce citrulline and nitric oxide, the latter of which plays an important role in cytotoxic mechanisms in myeloid cells and vasodilatation in endothelial cells [4], [34]. Alternatively, arginase I and arginase II metabolize L-Arg to L-ornithine and urea, the first being the precursor for the production of polyamines essential for cell proliferation and an important mechanism for detoxification of protein degradation in hepatocytes [65]. Two other enzymes, ADC and AGAT, convert L-Arg to agmatine, which, in turn, is converted to putrescine and urea by agmatinase [66]. Mammalian ADC is highly expressed in the brain [39], [120], while AGAT is expressed in the brain and heart [15], [37]. ADC and AGAT appear to be less important in the immune response.

The expression of arginase I and NOS2 in murine macrophages is differentially regulated by Th1 and Th2 cytokines [33], [68] with interferon gamma (IFN-γ) up-regulating NOS2 exclusively and IL-4, IL-10, and IL-13 inducing arginase I [67], [89]. The mitochondrial isoform arginase II is not significantly modulated by Th1 or Th2 cytokines [83]. In turn, arginase I and NOS2 appear to modulate each other's expression. The inhibition of arginase I leads to an increased NOS2 expression and, consequently, increases the production of NO [12]. Moreover, the upregulation of

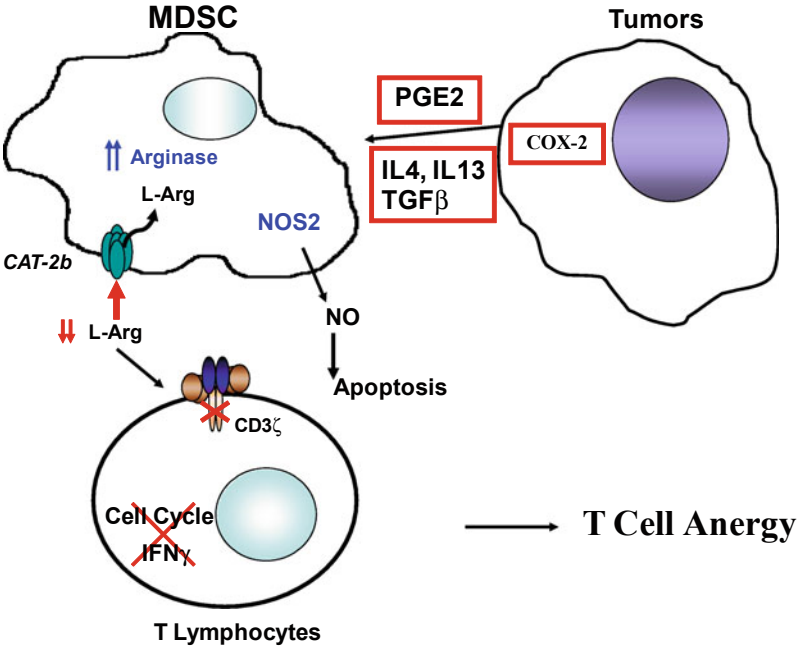

Fig. 13.2 T cell dysfunction induced by arginase I. Tumor cells expressing COX-2 and releasing PGE_2, and high levels of different mediators present in the tumor microenvironment including TGF-b, IL-4, and IL-13 induces the expression of arginase I and CAT-2B in MDSC. This leads to a reduction of extra cellular levels of L-Arginine, which finally activates GCN2 and blocks the expression of multiple genes including CD3 z, IFNg, cyclin D3 and cdk4. A similar reduction occurs in patients with cancer, but through the release of arginase into the extracellular environment. Furthermore, MDSC release NO, which is implicated in low recognition of antigens and direct induction of apoptosis in T cells.

arginase I inhibits NOS activity and contributes to the pathophysiology of several diseases including vascular dysfunction and asthma [119]. The mechanism of inhibition of NOS2 expression by arginase I appears to be mediated by L-Arg depletion, which blocks the translation of NOS2 [57]. In addition, low levels of nitric oxide induce nitrosylation of cysteine residues of arginase I which increases its biological activity, further reducing L-Arg [90].

The expression of arginase I or NOS2 also has effects on the extracellular levels of L-Arg. Peritoneal macrophages stimulated with IL-4 plus IL-13 increase the expression of arginase I and CAT-2B, which results in a rapid increase in the uptake of extracellular L-Arg with the consequent reduction of L-Arg in the microenvironment. In contrast, macrophages stimulated with IFN-γ that preferentially increase the expression of NOS2 do not increase CAT-2B expression and do not deplete L-Arg from the microenvironment [83]. Results from the arginase I and arginase II knockout mice confirm that only arginase I is able to deplete serum levels of L-Arg [16], [38]. Coculture experiments of macrophages producing arginase I and activated T cells

resulted in the loss of CD3ζ, an arrest in T-cell proliferation, and the inability of T cells to produce IFN-γ (but not IL-2). The addition of arginase inhibitors or exogenous L-Arg reversed the CD3ζ loss and reestablished T-cell proliferation [83]. These results were confirmed with macrophages from arginase I-conditional knockout mice (unpublished findings). In contrast, T cells cocultured with macrophages expressing NOS2 did not develop these alterations.

4 Effects of L-Arg Starvation on T Cells

The association between an increased expression of arginase I, a decrease in L-Arg levels, and changes in T-cell responses was first suggested by experiments showing that mice undergoing extensive surgery developed thymic involution and a decrease in splenic T cells. This effect was prevented by the injection of L-Arg [5]. Our initial experiments demonstrated that culturing T cells in a tissue culture medium with L-Arg levels $< 50\ \mu M$ resulted in a significant decrease in cell proliferation [104]. In addition, T cells activated in an L-Arg-free environment developed all the alterations previously described in tumor-bearing mice and cancer patients, i.e., the decreased expression of CD3ζ, an inability to upregulate Jak-3, a decreased translocation of NF-κB–p65, and the inability to produce IFN-γ [116]. More importantly, Rodriguez et al. also showed that the absence of L-Arg arrested T cells in the G_0–G_1 phase of the cell cycle, while T cells cultured with L-Arg progressed easily through the S and G_2–M phases [86]. This arrest in cell cycle progression was caused by a selective inability to upregulate cyclin D3 and cdk4, which did not affect other cyclin proteins [86]. In fact, silencing cyclin D3 in T cells resulted in a similar inhibition of proliferation as that caused by the absence of L-Arg. Additional research showed that L-Arg starvation impaired the expression of cyclin D3 and cdk4 in T cells through a decreased mRNA stability and diminished rate of translation [86], [88].

How does the depletion of one amino acid, L-Arg, leads to the specific molecular changes that result in T-cell anergy? Previous work had shown that amino acid starvation leads to the accumulation of empty aminoacyl-transfer RNAs (aminoacyl-tRNAs), which leads to the activation of general control nonrepressed 2 (GCN2) kinase that, in turn, phosphorylates the translation initiation factor eukaryotic initiation factor 2 alpha (eIF2α). The phosphorylated form of eIF2α binds with high affinity to eIF2β, blocking its ability to exchange guanosine diphosphate (GDP) with guanosine triphosphate (GTP), which inhibits the binding of the eIF2 complex to methionine-aminoacyl-tRNA. This results in a decreased initiation of global protein synthesis. Our results show that T cells cultured in a medium without L-Arg have high levels of phospho-eIF2α and a decreased global protein translation that preferentially impairs the synthesis and expression of the RNA-binding protein HuR, which confers stability to messenger RNA (mRNA) containing AUUA-rich elements such as cyclin D3 [86], [88]).

5 Arginase I Expression in Tumors-Infiltrating MDSCs

Some tumor cell lines including non-small lung carcinoma and breast carcinoma have been shown to express arginase [10], [95], [103]. This was thought to be a mechanism for the production of polyamines needed to sustain the rapid proliferation of tumor cells. Our results suggest instead that arginase I is primarily expressed in MDSCs infiltrating tumors, which inhibits T-cell function and represents a potent mechanism for stromal remodeling and for tumor evasion [84].

Two major subsets of MDSCs have been reported: granulocytic MDSCs (G-MDSCs) that are $CD11b^{+}$ $LY6G^{+}$ $LY6C^{low}$ and monocytic MDSCs (M-MDSCs) that are $CD11b^{+}$ $LY6G^{-}$ $LY6C^{high}$ [111]. However, several reports have also shown the presence of tumor-infiltrating MDSCs that express $CD11b^{+}$ $LY6G^{-}$ $LY6C^{-}$, a phenotype reminiscent of alternatively activated macrophages [17], [84]. In addition, granulocytic and monocytic subpopulations of iMCs can be found in the bone marrow of healthy mice, but these do not appear to suppress T-cell function [8], [53], [97]. These variations in MDSC phenotype appear to be the result of the different combinations of soluble factors produced by different tumor types. The balance between G-MDSCs and M-MDSCs, their biology, and the mechanisms that lead to their accumulation is still a matter of significant research. Recent data suggest that M-MDSCs may be precursors of G-MDSCs [112]. G-MDSCs are the major source of arginase I in tumor-bearing hosts and are significantly more potent inhibitors of T-cell function in vitro [84], whereas tumor-associated M-MDSCs primarily metabolize L-Arg through NOS2 [111]. In addition, recent publications demonstrate that MDSCs promote angiogenesis and create a "pre-metastatic niche" for circulating tumor cells [27].

In spite of the phenotypic differences, researchers have shown that the depletion of both G- and M-MDSCs using antibodies against the myeloid differentiation antigen GR-1 anti-GR-1) antibodies induces an antitumor effect mediated by $CD8^{+}$ T cells [35], [80], [93].

6 Molecular Mechanisms of Tolerance Induced by MDSCs

The mechanisms by which MDSCs induce T-cell tolerance include the production of arginase I, peroxynitrites, or ROS (H_2O_2). The effect of arginase I does not require cell-to-cell contact, while peroxynitrites and H_2O_2 require close proximity of MDSCs and T cells. Our data suggest that the depletion of extracellular L-Arg by arginase I represents one of the primary mechanisms for the induction of T-cell tolerance [83]–[85]. In fact, the depletion of L-Arg through these mechanisms is not limited to the tumor microenvironment but can also be measured in the depletion of L-Arg levels in the plasma of patients with renal cell carcinoma [87], [117]. Furthermore, the addition of arginase I inhibitors nor-N(omega)-hydroxy-L-arginine (nor-NOHA) or NOHA in vitro, or its injection in tumor-bearing mice, prevents the loss of T-cell function and results in an immune-mediated antitumor response which inhibits tumor growth in a dose-dependent manner [84].

Peroxynitrites and H_2O_2 also produced by MDSCs cause T-cell tolerance through cell-to-cell contact. This mechanism appears to require the coexpression of arginase I and NOS2 [7], as shown by the fact that the addition of NOS2 and arginase inhibitors to cocultures of MDSCs and activated T cells completely reestablishes T-cell function [9]. It is possible that this cell–cell suppression of T-cell function is primarily mediated by the production of peroxynitrites. Under limiting amounts of L-Arg, NOS2 preferentially produces peroxynitrites ($ONOO^-$) instead of nitric oxide (NO). $ONOO^-$ are highly reactive oxidizing agents that nitrate proteins and induce T-cell apoptosis [42]. Nytosylation also appears to affect the conformational flexibility of the T-cell antigen receptor and its interaction with the major histocompatibility complex (MHC) in $CD8^+$ cells. Therefore, MDSCs can directly disrupt the binding of specific peptides on MHC to $CD8^+$ T cells [58], [73]. MDSCs coexpressing arginase I and NOS2 can also impair $CD8^+$ T-cell function by blocking their ability to secrete IFN-γ when stimulated with specific antigens [23], [51], [53], [84], [97], [106]. This suppression requires the production of IL-13 and IFN-γ [25], [96], [97] and signaling through the STAT1 transcription factor [51]. In addition, MDSCs have been shown to induce regulatory T cells by producing high levels of stem cell factor (SCF) [36], [79].

7 MDSCs in Human Tumors

Human MDSC phenotypes vary significantly ranging from iMCs [94], [100] to activated granulocytes [87]. In cancer patients, M-MDSCs have been characterized as expressing either $CD14^+HLA\text{-}DR^{lo}$ or $CD11b^+CD14^-CD33^+CD15^-$, while G-MDSCs express $CD11b^+CD14^-CD33^+CD15^+CD66b^+$ [24], [30]. A study of 117 patients with metastatic renal cell carcinoma (RCC) demonstrated a six- to tenfold increase in arginase activity in the peripheral blood mononuclear cells (PBMCs), as compared to normal controls [117]. Separation of the different subpopulations of MDSCs demonstrated that the major source of arginase were G-MDSC which separated with the PBMCs when centrifuged over Ficoll–Hypaque [87]. These cells suppressed the ability of T cells to proliferate and produce IFN-γ in vitro.

Human MDSCs differ in several ways from murine MDSCs. Normal human granulocytes constitutively express arginase I as a potent antibacterial and antiviral mechanism. Arginase I expression in mature human granulocytes does not appear to be upregulated by cytokines such as IL-4, IL-13, or TGF-β. However, hematopoietic stem cells can be induced to express high levels of arginase I when cultured in a medium with GM-CSF, G-CSF, and IL-6 [59]. Human MDSCs also differ from murine MDSCs in their mechanism of arginine depletion. Human MDSCs do not uptake L-Arg (as murine MDSCs do). Instead, arginase I is stored in primary [69] or gelatinase granules [40] and is released into the microenvironment at the time of degranulation, depleting the local levels of L-Arg. T cells stimulated in this L-Arg-depleted microenvironment develop a loss of the CD3ζ chain expression and are unable to produce IFN-γ and to proliferate [47], [70], [117]. In fact, the high levels

of arginase I released in the sera of renal cell carcinoma patients result in a decrease of L-Arg to < 50 μM (normal control levels are 50–150 μM) and a significant increase in ornithine levels, a result of arginine metabolism by arginase I. Therefore, high arginase I levels have a systemic metabolic effect (L-Arg depletion) and block the protective T-cell responses [117].

8 Generation of MDSCs from Hematopoietic Progenitors

The process of myelopoiesis and commitment to a myeloid-cell lineage is tightly regulated. Accumulating evidence however indicates that tumor-derived factors alter this process and result in an increased number of iMCs, the majority of which are MDSCs [24]. Different cytokines including VEGF and GM-CSF participate in the recruitment of MDSCs from the bone marrow, [17]. In fact, reports by Ohm and Carbone show that serum levels of VEGF directly correlated with numbers of MDSCs in the blood and spleen and are associated with poor prognosis in cancer patients [74]–[76]. Tumor-derived VEGF has been previously associated with an arrest in dendritic cell maturation through the inhibition of NF-κB signaling [22], [78]. Treatment of MDSCs with all-*trans* retinoic acid appears to counter the inhibition of NF-κB signaling and promote MDSC differentiation into mature APCs [52]. Interestingly, however, treatment of patients with IL-2 and the VEGF-R inhibitor, becavizumab, resulted in an increase in MDSCs [87].

Increased levels of GM-CSF have also been associated with MDSC-dependent suppression which was reversed by the use of neutralizing antibodies to GM-CSF [8]. Similar effects on MDSCs have been suggested with other growth factors including Fms-like tyrosine kinase 3 (Flt3) ligand [99], stem cell factor (SCF) [79], and S100 calcium-binding protein A9 (S100A9) [11]. In a recent study, Youn et al. demonstrated in a series of elegant experiments that M-MDSCs from tumor-bearing mice were able to acquire a granulocytic morphology in the presence of tumor cell-conditioned medium in vitro or after the adoptive transfer to tumor-bearing recipients, effectively converting into G-MDSCs. This process appeared to be controlled by epigenetic silencing of the retinoblastoma gene through modifications mediated by histone deacetylase 2 (HDAC-2) [112]. Another study suggested instead that G-CSF, GM-CSF, and IL-6 are the central mediators of the maturation of hematopoietic progenitors into MDSCs [59]. A complete understanding of these pathways could identify new molecular targets aimed at blocking MDSC maturation.

9 Induction of Arginase and Other Suppressive Mechanisms in MDSCs

In vitro studies had shown that murine macrophages cultured with IL-4 + IL-13 (and TGF-β) increased the expression of arginase I and their ability to suppress T cells. We explored whether these factors were being produced by tumors and

whether they induced arginase I in MDSCs in vivo. Experiments using 3LL Lewis lung carcinoma and Colon carcinoma cell line MCA-38 however failed to show the production of these cytokines by these cell lines. Instead, what we found was the expression of high levels of cyclooxygenase-2 (COX-2) and the production of high quantities of prostanoids including PGE_2. COX-2 inhibitors or silencing of COX-2 in tumor cells completely blocked their ability to induce arginase I in MDSCs [85]. Consequently, treatment of tumor-bearing mice with COX-2 inhibitor sc-58125 decreased the expression of arginase I in MDSCs infiltrating the tumor and induced an immune-mediated antitumor effect [85]. Similar results have been reported in mice bearing the 4T1 breast carcinoma [98] and in mice with 1,2-dimethylhydrazine diHCl (1,2-DMH)-induced colon carcinoma [105]. Other factors may also play a role in the induction of arginase in MDSCs including hypoxia-inducible factor 1 (HIF-1) and HIF-2 (reviewed in [92]), IL-4, IL-13, and IFN-γ [25] in mice and IL-8 in human MDSCs (Rotondo et al). In addition, CCAAT enhancer-binding protein beta (C/EBPβ) [59] and STAT3 [108] have been proposed as molecular regulators of arginase in tumors.

10 Inhibition of MDSCs in vivo

Blocking the accumulation of MDSCs in tumor-bearing hosts has been achieved in animal models or patients with antibodies against Gr-1, CD11b, and CSF1, inhibitors of CSF1 receptor (CSFR1/c-fms), and the multi-targeted receptor tyrosine kinase inhibitor sunitinib. In addition, the use of the antimetabolites gemcitabine (GEM) and 5-fluorouracil (5-FU) has also shown the ability to deplete MDSCs and partially restore T-cell function in tumor-bearing hosts [21], [44], [54], [71], [72], [79]. However, the effects of these anti-MDSC approaches on specific MDSC subpopulations remain unclear. Preliminary data suggest that sunitinib may block the proliferation of M-MDSCs and impair the survival of G-MDSCs [44]. A goal of targeted depletion of selective MDSC subpopulations, or the silencing of specific suppressive mechanisms from MDSCs, may allow us to enhance the efficacy of immunotherapy and other forms of cancer treatment.

11 MDSCs: Lessons from Other Diseases and Future Applications

MDSCs are not unique to cancer. Trauma patients and patients with chronic infections including active pulmonary tuberculosis also have increased numbers of MDSCs expressing arginase I that inhibit T-cell function. These data suggest that MDSCs may represent a normal process triggered by tissue damage (danger signal) with the aim of protecting the integrity of the tissues and "healing" the initial injury. This mechanism was described in the late 1980s by Albina et al. studying the healing

of surgical wounds [1]. They described that the tissue surrounding a surgical wound was initially infiltrated by cells expressing iNOS which would most likely eliminate microbial agents contaminating the wound. This surge was followed by cells expressing arginase I which metabolize L-Arg to ornithine, which, in turn, would trigger the synthesis of collagen by fibroblasts, ultimately leading to the healing of the surgical wound. The local depletion of L-Arg would also prevent T cells from infiltrating a healing tissue and cause chronic inflammation at the site. In cancer or chronic infections, tissue damage would also trigger a similar response with the proliferation of fibroblasts producing collagen, aimed at isolating and healing the damaged tissue (i.e., malignant growth). As a matter of fact, many tumors are surrounded by dense fibrous tissue that makes its surgical excision difficult. The major difference between both disease processes (surgical wound vs malignant tumor) is that the surgical wounds heal, thus ending the role for arginase-producing MDSCs. In contrast, malignant tumors do not stop growing and destroying tissue (would not "heal") promoting instead a chronic inflammatory process mediated by MDSCs. The continuous production of arginase I would ultimately lead to the depletion of L-Arg from the microenvironment and the development of T-cell anergy. Therefore, our working hypothesis has been that tumors "hijack" a normal healing process by promoting the differentiation and activation of MDSCs expressing arginase I, which not only creates a nurturing stroma for the tumor cells but also inhibits any protective antitumor T-cell response. Although this is likely to be an oversimplified version of the complex mechanisms triggered in vivo, it provides a model to understand a complex event in the development of cancer, which could enable the design of new therapeutic approaches to interrupt this dysfunctional response.

In summary, the role of MDSCs in the development of malignant tumors has clearly been demonstrated over the past two decades. The mechanisms that induce these immunosuppressive cells are primarily produced in the tumor microenvironment and include cytokines such as IL-4, IL-13, or TGF-β, or prostaglandins (Fig. 13.2). The combination of factors is likely to vary between the different types of tumors. However, understanding how these factors stimulate the maturation of MDSCs and the molecular mechanisms that regulate their function should help develop new targeted therapies to inhibit MDSCs and enhance the efficacy of cancer therapies.

References

1. Albina JE, Caldwel MD, Henry WL Jr, Mills CD (1989) Regulation of macrophage functions by L-arginine. J Exp Med 169:1021–1029
2. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, Carbone DP, Gabrilovich DI (2000) Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res 6:1755–1766
3. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, Carbone DP, Gabrilovich DI (2001) Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 166:678–689

4. Amber IJ, Hibbs JB Jr, Parker CJ, Johnson BB, Taintor RR, Vavrin Z (1991) Activated macrophage conditioned medium: identification of the soluble factors inducing cytotoxicity and the L-arginine dependent effector mechanism. J Leukoc Biol 49:610–620
5. Barbul A, Rettura G, Levenson SM, Seifter E (1977). Arginine: a thymotropic and wound-healing promoting agent. Surg. Forum 28:101–103
6. Bronstein-Sitton N, Cohen-Daniel L, Vaknin I, Ezernitchi AV, Leshem B, Halabi A, Houri-Hadad Y, Greenbaum E, Zakay-Rones Z, Shapira L, Baniyash M (2003) Sustained exposure to bacterial antigen induces interferon-gamma-dependent T cell receptor zeta down-regulation and impaired T cell function. Nat Immunol 4:957–964
7. Bronte V, Zanovello P (2005) Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 5:641–654
8. Bronte V, Apolloni E, Cabrelle A, Ronca R, Serafini P, Zamboni P, Restifo NP, Zanovello P (2000) Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. Blood 96:3838–3846
9. Bronte V, Serafini P, De Santo C, Marigo I, Tosello V, Mazzoni A, Segal DM, Staib C, Lowel M, Sutter G, Colombo MP, Zanovello P (2003) IL-4-Induced Arginase 1 Suppresses Alloreactive T Cells in Tumor-Bearing Mice. J Immunol 170:270–278
10. Chang CI, Liao JC, Kuo L (2001) Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. Cancer Res 61:1100–1106
11. Cheng P, Corzo CA, Luetteke N, Yu B, Nagaraj S, Bui MM, Ortiz M, Nacken W, Sorg C, Vogl T, Roth J, Gabrilovich DI (2008) Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med 205:2235–2249
12. Chicoine LG, Paffet ML, Young TL, Nelin LD (2004) Arginase inhibition increases nitric oxide production in bovine pulmonary arterial endothelial cells. Am J Physiol Lung Cell Mol Physiol 287:L60–L68
13. Clos EI, Simon A, Vekony N, Rotman A (2004) Plasma membrane transporters for arginine. J Nutr 134:2752S–2759S
14. Corsi MM, Maes HH, Wasserman K, Fulgenzi A, Gaja G, Ferrero ME (1998) Protection by L-2-oxothiazolidine-4-carboxylic acid of hydrogen peroxide-induced CD3zeta and CD16zeta chain down-regulation in human peripheral blood lymphocytes and lymphokine-activated killer cells. Biochem Pharmacol 56:657–662
15. Cullen ME, Yuen AH, Felkin LE, Smolenski RT, Hal JL, Grindle S, Miller LW, Birks EJ, Yacoub MH, Barton PJ (2006) Myocardial expression of the arginine: glycine amidinotransferase gene is elevated in heart failure and normalized after recovery: potential implications for local creatine synthesis. Circulation 114:I16–I20
16. Deignan JL, Livesay JC, Yo PK, Goodman SI, O'Brien WE, Iyer RK, Cederbaum SD, Grody WW (2006). Ornithine deficiency in the arginase double knockout mouse. Mol Genet Metab 89:87–96
17. Dolcetti L, Peranzoni E, Ugel S, Marigo I, Fernandez GA, Mesa C, Geilich M, Winkels G, Traggiai E, Casati A, Grassi F, Bronte V (2010) Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. Eur J Immunol 40:22–35
18. Dong H, Zhu G, Tamada K, Chen L (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5:1365–1369
19. Finke JH, Zea AH, Stanley J, Longo DL, Mizoguchi H, Tubbs RR, Wiltrout RH, O'Shea JJ, Kudoh S, Klein E, Ochoa AC (1993) Loss of T-cell receptor zeta chain and p56 lck in T-cells infiltrating human renal cell carcinoma. Cancer Res 53:5613–5616
20. Gabrilovich D (2004) Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol 4:941–952
21. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9:162–174
22. Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP (1998) Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood 92:4150–4166

23. Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM (2001) Mechanism of immune dysfunction in cancer mediated by immature Gr-1 + myeloid cells. J Immunol 166:5398–5406
24. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12:253–268
25. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, Basso G, Brombacher F, Borrello I, Zanovello P, Bicciato S, Bronte V (2006) Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8 + T cells. J Clin Invest 116:2777–2790
26. Gattinoni L, Powel DJ Jr, Rosenberg SA, Restifo NP (2006) Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol 6:383–393
27. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R (2011) Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell 20:300–314
28. Greenberg PD, Cheever MA (1984) Treatment of disseminated leukemia with cyclophosphamide and immune cells: tumor immunity reflects long-term persistence of tumor-specific donor T cells. J Immunol 133:3401–3407
29. Greenberg PD, Cheever MA, Fefer A (1981) Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1+2- lymphocytes. J Exp Med 154:952–963
30. Greten TF, Manns MP, Korangy F (2011) Myeloid derived suppressor cells in human diseases. Int Immunopharmacol 11:802–807
31. Hellstrom I, Sjogren HO, Warner G, Hellstrom KE (1971) Blocking of cell-mediated tumor immunity by sera from patients with growing neoplasms. Int J Cancer 7:226–237
32. Hellstrom KE, Hellstrom I, Nelson K (1983) Antigen-specific suppressor ("blocking") factors in tumor immunity. Biomembranes 11:365–388
33. Hesse M, Modolel M, La Flamme AC, Schito M, Fuentes JM, Cheever AW, Pearce EJ, Wyn TA (2001) Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. J Immunol 167:6533–6544
34. Hibbs JB Jr, Taintor RR, Vavrin Z (1987) Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science 235:473–476
35. Holda JH, Maier T, Claman HN (1985) Murine graft-versus-host disease across minor barriers: immunosuppressive aspects of natural suppressor cells. Immunol Rev 88:87–105
36. Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, Divino CM, Chen SH (2006) Gr-1+CD115+immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res 66:1123–1131
37. Item CB, Stockler-Ipsiroglu S, Stromberger C, Muhl A, Alessandri MG, Bianchi MC, Tosetti M, Fornai F, Cioni G (2001) Arginine: glycine amidinotransferase deficiency: the third inborn error of creatine metabolism in humans. Am J Hum Genet 69:1127–1133
38. Iyer RK, Yoo PK, Kern RM, Rozengurt N, Tsoa R, O'Brien WE, Yu H, Grody WW, Cederbaum SD (2002) Mouse model for human arginase deficiency. Mol Cell Biol 22:4491–4498
39. Iyo AH, Zhu MY, Ordway GA, Regunathan S (2006) Expression of arginine decarboxylase in brain regions and neuronal cells. J Neurochem 96:1042–1050
40. Jacobsen LC, Theilgaard-Monch K, Christensen EI, Borregaard N (2007) Arginase 1 is expressed in myelocytes/metamyelocytes and localized in gelatinase granules of human neutrophils. Blood 109:3084–3087
41. Jenkins MK, Chen CA, Jung G, Mueller DL, Schwartz RH (1990) Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. J Immunol 144:16–22
42. Kasic T, Colombo P, Soldani C, Wang CM, Miranda E, Roncalli M, Bronte V, Viola A (2011) Modulation of human T-cell functions by reactive nitrogen species. Eur J Immunol 41:1843–1849
43. Klausner RD, Lippincott-Schwartz J, Bonifacino JS (1990) The T cell antigen receptor: insights into organelle biology. Annu Rev Cell Biol 6:403–431
44. Ko JS, Rayman P, Ireland J, Swaidani S, Li G, Bunting KD, Rini B, Finke JH, Cohen PA (2010) Direct and differential suppression of myeloid-derived suppressor cell subsets by sunitinib is compartmentally constrained. Cancer Res 70:3526–3536

45. Kono K, Ressing ME, Brandt RM, Melief CJ, Potkul RK, Andersson B, Petersson M, Kast WM, Kiessling R (1996a) Decreased expression of signal-transducing zeta chain in peripheral T cells and natural killer cells in patients with cervical cancer. Clin Cancer Res 2:1825–1828
46. Kono K, Salazar-Onfray F, Petersson M, Hansson J, Masucci G, Wasserman K, Nakazawa T, Anderson P, Kiessling R (1996b) Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell-and natural killer cell-mediated cytotoxicity. Eur J Immunol 26:1308–1313
47. Kropf P, Baud D, Marshal SE, Munder M, Mosley A, Fuentes JM, Bangham CR, Taylor GP, Herath S, Choi BS, Soler G, Teoh T, Modolel M, Muller I (2007) Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. Eur J Immunol 37:935–945
48. Krummel MF, Allison JP (1995) CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med 182:459–465
49. Kryczek I, Wei S, Zou L, Zhu G, Mottram P, Xu H, Chen L, Zou W (2006a) Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. J Immunol 177:40–44
50. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, Brumlik M, Cheng P, Curiel T, Myers L, Lackner A, Alvarez X, Ochoa A, Chen L, Zou W (2006b) B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med 203:871–881
51. Kusmartsev S, Gabrilovich DI (2005) STAT1 signaling regulates tumor-associated macrophage-mediated T cell deletion. J Immunol 174:4880–4891
52. Kusmartsev S, Cheng F, Yu B, Nefedova Y, Sotomayor E, Lush R, Gabrilovich D (2003) All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. Cancer Res 63:4441–4449
53. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI (2004) Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. J Immunol 172:989–999
54. Kusmartsev S, Su Z, Heiser A, Dannul J, Eruslanov E, Kubler H, Yancey D, Dahm P, Vieweg J (2008) Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. Clin Cancer Res 14:8270–8278
55. Kus I, Saito T, Johnson JT, Whiteside TL (1999) Clinical significance of decreased zeta chain expression in peripheral blood lymphocytes of patients with head and neck cancer. Clin Cancer Res 5:329–334
56. Lala PK, Parhar RS, Singh P (1986) Indomethacin therapy abrogates the prostaglandin-mediated suppression of natural killer activity in tumor-bearing mice and prevents tumor metastasis. Cell Immunol 99:108–118
57. Lee J, Ryu H, Ferrante RJ, Morris SM Jr, Ratan RR (2003) Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. Proc Natl Acad Sci U S A 100:4843–4848
58. Li F, Lu J, Ma X (2011) Metabolomic screening and identification of the bioactivation pathways of ritonavir. Chem Res Toxicol 24:2109–2114
59. Marigo I, Bosio E, Solito S, Mesa C, Fernandez A, Dolcetti L, Ugel S, Sonda N, Bicciato S, Falisi E, Calabrese F, Basso G, Zanovello P, Cozzi E, Mandruzzato S, Bronte V (2010) Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. Immunity 32:790–802
60. McHugh RS, Shevach EM (2002) Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. J Immunol 168:5979–5983
61. McHugh RS, Shevach EM, Margulies DH, Natarajan K (2001) A T cell receptor transgenic model of severe, spontaneous organ-specific autoimmunity. Eur J Immunol 31:2094–2103
62. Miescher S, Whiteside TL, Carrel S, von Fliedner V (1986) Functional properties of tumor-infiltrating and blood lymphocytes in patients with solid tumors: effects of tumor cells and their supernatants on proliferative responses of lymphocytes. J Immunol 136:1899–1907

63. Miescher S, Stoeck M, Qiao L, Barras C, Barrelet L, von Fliedner V (1988) Preferential clonogenic deficit of CD8-positive T-lymphocytes infiltrating human solid tumors. Cancer Res 48:6992–6998
64. Mizoguchi H, O'Shea JJ, Longo DL, Loeffler CM, McVicar DW, Ochoa AC (1992) Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. Science 258:1795–1798
65. Morris SM Jr (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 22:87–105
66. Morris SM Jr (2012). Arginases and arginine deficiency syndromes. Curr Opin Clin Nutr Metab Care 15:64–70
67. Munder M, Eichman K, Modolel M (1998) Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4 + T cells correlates with Th1/Th2 phenotype. J Immunol 160:5347–5354
68. Munder M, Eichman K, Moran JM, Centeno F, Soler G, Modolel M (1999) Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. J Immunol 163:3771–3777
69. Munder M, Mollinedo F, Calafat J, Canchado J, Gil-Lamaignere C, Fuentes JM, Luckner C, Doschko G, Soler G, Eichman K, Muller FM, Ho AD, Goerner M, Modolel M (2005) Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood 105:2549–2556
70. Munder M, Schneider H, Luckner C, Giese T, Langhans CD, Fuentes JM, Kropf P, Mueller I, Kolb A, Modolel M, Ho AD (2006) Suppression of T-cell functions by human granulocyte arginase. Blood 108:1627–1634
71. Mundy-Bosse BL, Lesinski GB, Jaime-Ramirez AC, Benninger K, Khan M, Kuppusamy P, Guenterberg K, Kondadasula SV, Chaudhury AR, La Perle KM, Kreiner M, Young G, Guttridge DC, Carson WE III (2011a) Myeloid-derived suppressor cell inhibition of the IFN response in tumor-bearing mice. Cancer Res 71:5101–5110
72. Mundy-Bosse BL, Young GS, Bauer T, Binkley E, Bloomston M, Bil MA, Bekaii-Saab T, Carson WE III, Lesinski GB (2011b) Distinct myeloid suppressor cell subsets correlate with plasma IL-6 and IL-10 and reduced interferon-alpha signaling in CD4(+) T cells from patients with GI malignancy. Cancer Immunol Immunother 60:1269–1279
73. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, Herber DL, Schneck J, Gabrilovich DI (2007) Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat Med 13:828–835
74. Ohm JE, Carbone DP (2001a) VEGF as a mediator of tumor-associated immunodeficiency. Immunol Res 23:263–272
75. Ohm JE, Carbone DP (2001b) VEGF as a mediator of tumor-associated immunodeficiency. Immunol Res 23:263–272
76. Ohm JE, Shurin MR, Esche C, Lotze MT, Carbone DP, Gabrilovich DI (1999) Effect of vascular endothelial growth factor and FLT3 ligand on dendritic cell generation in vivo. J Immunol 163:3260–3268
77. Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T (1996) Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T- cell responses. Proc Natl Acad Sci U S A 93:13119–13124
78. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, Gabrilovich DI (1998) Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J Immunol 160:1224–1232
79. Pan PY, Wang GX, Yin B, Ozao J, Ku T, Divino CM, Chen SH (2008) Reversion of immune tolerance in advanced malignancy: modulation of myeloid derived suppressor cell development by blockade of SCF function. Blood 111:219–228
80. Pekarek LA, Star BA, Toledano AY, Schreiber H (1995) Inhibition of tumor growth by elimination of granulocytes. J Exp Med 181:435–440
81. Rabinowich H, Reichert TE, Kashi Y, Gastman BR, Bel MC, Whiteside TL (1998). Lymphocyte apoptosis induced by Fas li. J Clin Invest 101:2579–2588

82. Rodgers S, Rees RC, Hancock BW (1994) Changes in the phenotypic characteristics of eosinophils from patients receiving recombinant human interleukin-2 (rhIL-2) therapy. Br J Haematol 86:746–753
83. Rodriguez PC, Zea AH, DeSalvo J, Culotta KS, Zabaleta J, Quiceno DG, Ochoa JB, Ochoa AC (2003) L-arginine consumption by macrophages modulates the expression of CD3zeta chain in T lymphocytes. J Immunol 171:1232–1239
84. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, Delgado A, Correa P, Brayer J, Sotomayor EM, Antonia S, Ochoa JB, Ochoa AC (2004) Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 64:5839–5849
85. Rodriguez PC, Hernandez CP, Quiceno D, Dubinet SM, Zabaleta J, Ochoa JB, Gilbert J, Ochoa AC (2005) Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med 202:931–939
86. Rodriguez PC, Quiceno DG, Ochoa AC (2007) L-arginine availability regulates T-lymphocyte cell-cycle progression. Blood 109:1568–1573
87. Rodriguez PC, Ernstof MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, Ochoa AC (2009) Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res 69:1553–1560
88. Rodriguez PC, Hernandez CP, Morrow K, Sierra R, Zabaleta J, Wyczechowska DD, Ochoa AC (2010) L-arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. J Immunol 185:5198–5204
89. Rutschman R, Lang R, Hesse M, Ihle JN, Wyn TA, Murray PJ (2001) Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. J Immunol 166:2173–2177
90. Santhanam L, Lim HK, Lim HK, Miriel V, Brown T, Patel M, Balanson S, Ryo S, Anderson M, Irani K, Khanday F, Di CL, Nyhan D, Hare JM, Christianson DW, Rivers R, Shoukas A, Berkowitz DE (2007) Inducible NO synthase dependent S-Nitrosylation and activation of Arginase1 contribute to age-related endothelial dysfunction. Circ Res 101:692–702
91. Schmielau J, Fin OJ (2001) Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. Cancer Res 61:4756–4760
92. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732
93. Seung LP, Rowley DA, Dubey P, Schreiber H (1995) Synergy between T-cell immunity and inhibition of paracrine stimulation causes tumor rejection. Proc Natl Acad Sci U S A 92:6254–6258
94. Sica A, Bronte V (2007) Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117:1155–1166
95. Singh R, Pervin S, Karimi A, Cederbaum S, Chaudhuri G (2000) Arginase activity in human breast cancer cell lines: N(omega)-hydroxy-L- arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. Cancer Res 60:3305–3312
96. Sinha P, Clements VK, Ostrand-Rosenberg S (2005a) Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res 65:11743–11751
97. Sinha P, Clements VK, Ostrand-Rosenberg S (2005b) Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease. J Immunol 174:636–645
98. Sinha P, Clements VK, Fulton AM, Ostrand-Rosenberg S (2007) Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. Cancer Res 67:4507–4513
99. Solheim JC, Reber AJ, Ashour AE, Robinson S, Futakuchi M, Kurz SG, Hood K, Fields RR, Shafer LR, Cornel D, Sutjipto S, Zurawski S, LaFace DM, Singh RK, Talmadge JE (2007) Spleen but not tumor infiltration by dendritic and T cells is increased by intravenous adenovirus-Flt3 ligand injection. Cancer Gene Ther 14:364–371
100. Solito S, Falisi E, az-Montero CM, Doni A, Pinton L, Rosato A, Francescato S, Basso G, Zanovello P, Onicescu G, Garrett-Mayer E, Montero AJ, Bronte V, Mandruzzato S (2011) A

human promyelocytic-like population is responsible for the immune suppression mediated by myeloid-derived suppressor cells. Blood 118:2254–2265
101. Staveley-O'Carrol K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, Pardol D, Levitsky H (1998). Induction of antigen-specific T cell anergy: an early event in the course of tumor progression. Proc Natl Acad Sci U S A 95:1178–1183
102. Stevenson FK (2005) Update on cancer vaccines. Curr Opin Oncol 17:573–577
103. Suer GS, Yoruk Y, Cakir E, Yorulmaz F, Gulen S (1999) Arginase and ornithine, as markers in human non-small cell lung carcinoma. Cancer Biochem Biophys 17:125–131
104. Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS, Zea AH, Ochoa AC (2001) L-Arginine regulates the expression of the T-cell receptor zeta chain (CD3zeta) in Jurkat cells. Clin Cancer Res 7:958s–965s
105. Talmadge JE, Hood KC, Zobel LC, Shafer LR, Coles M, Toth B (2007) Chemoprevention by cyclooxygenase-2 inhibition reduces immature myeloid suppressor cell expansion. Int Immunopharmacol 7:140–151
106. Van Ginderachter JA, Meerschaut S, Liu Y, Brys L, De GK, Hassanzadeh GG, Raes G, De BP (2006) Peroxisome proliferator-activated receptor gamma (PPARgamma) ligands reverse CTL suppression by alternatively activated (M2) macrophages in cancer. Blood 108:525–535
107. Varesio L, Giovarelli M, Landolfo S, Forni G (1979) Suppression of proliferative response and lymphokine production during the progression of a spontaneous tumor. Cancer Res 39: 4983–4988
108. Vasquez-Dunddel D, Pan F, Zeng Q, Gorbounov M, Albesiano E, Fu J, Blosser RL, Tam AJ, Bruno T, Zhang H, Pardol D, Kim Y (2013) STAT3 regulates arginase-I in myeloid-derived suppressor cells from cancer patients. J Clin Invest 123:1580–1589
109. Whiteside TL, Rabinowich H (1998) The role of Fas/FasL in immunosuppression induced by human tumors. Cancer Immunol Immunother 46:175–184
110. Whiteside TL, Miescher S, Moretta L, von Flidner V (1988) Cloning and proliferating precursor frequencies of tumor-infiltrating lymphocytes from human solid tumors. Transplant Proc 20:342–343
111. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI (2008) Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 181:5791–5802
112. Youn JI, Kumar V, Collazo M, Nefedova Y, Condamine T, Cheng P, Villagra A, Antonia S, McCaffrey JC, Fishman M, Sarnaik A, Horna P, Sotomayor E, Gabrilovich DI (2013) Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer. Nat Immunol 14:211–220
113. Zarour H, De SC, Lehman F, Marchand M, Lethe B, Romero P, Boon T, Renauld JC (1996) The majority of autologous cytolytic T-lymphocyte clones derived from peripheral blood lymphocytes of a melanoma patient recognize an antigenic peptide derived from gene Pmel17/gpJ. Invest Dermatol 107:63–67
114. Zea AH, Curti BD, Longo DL, Alvord WG, Strobl SL, Mizoguchi H, Creekmore SP, O'Shea JJ, Powers GC, Urba WJ, and (1995) Alterations in T cell receptor and signal transduction molecules in melanoma patients. Clin Cancer Res 1:1327–1335
115. Zea AH, Ochoa MT, Ghosh P, Longo DL, Alvord WG, Valderrama L, Falabella R, Harvey LK, Saravia N, Moreno LH, Ochoa AC (1998) Changes in expression of signal transduction proteins in T lymphocytes of patients with leprosy. Infect Immun 66:499–504
116. Zea AH, Rodriguez PC, Culotta KS, Hernandez CP, DeSalvo J, Ochoa JB, Park HJ, Zabaleta J, Ochoa AC (2004) l-Arginine modulates CD3zeta expression and T cell function in activated human T lymphocytes. Cell Immunol 232:21–31
117. Zea AH, Rodriguez PC, Atkins MB, Hernandez C, Signoretti S, Zabaleta J, McDermot D, Quiceno D, Youmans A, O'Neil A, Mier J, Ochoa AC (2005) Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. Cancer Res 65:3044–3048
118. Zea AH, Culotta KS, Ali J, Mason C, Park HJ, Zabaleta J, Garcia LF, Ochoa AC (2006) Decreased expression of CD3zeta and nuclear transcription factor kappa B in patients with

pulmonary tuberculosis: potential mechanisms and reversibility with treatment. J Infect Dis 194:1385–1393
119. Zhang C, Hein TW, Wang W, Miller MW, Fossum TW, McDonald MM, Humphrey JD, Kuo L (2004) Upregulation of vascular arginase in hypertension decreases nitric oxide-mediated dilation of coronary arterioles. Hypertension 44:935–943
120. Zhu MY, Iyo A, Piletz JE, Regunathan S (2004) Expression of human arginine decarboxylase, the biosynthetic enzyme for agmatine. Biochim Biophys Acta 1670:156–164