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## Metabolism of L-Arginine by Myeloid-Derived Suppressor Cells in Cancer: Mechanisms of T cell suppression and Therapeutic Perspectives

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### Abstract

Patients with cancer have an impaired T cell response that can decrease the potential therapeutic benefit of cancer vaccines and other forms of immunotherapy. The establishment of a chronic inflammatory environment in patients with cancer plays a critical role in the induction of T cell dysfunction. The accumulation of myeloid-derived suppressor cells (MDSC) in tumor bearing hosts is a hallmark of malignancy-associated inflammation and a major mediator of the induction of T cell suppression in cancer. Recent findings in tumor bearing mice and cancer patients indicate that the increased metabolism of L-Arginine (L-Arg) 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.

### Keywords

MDSC; tumor immunology; arginine

### INTRODUCTION

Modern concepts in carcinogenesis including the viral etiology of certain malignancies, the presence of mutated onco-proteins, and the over-expression of certain normal antigens, support the concept that antigen specific immune responses can be generated to control tumor growth. However, three decades of clinical trials in cancer immunotherapy have also made evident that tumor cells develop sophisticated mechanisms to evade the immune response. Extensive research has characterized multiple pathways triggered by the tumor microenvironment that result in the impairment of T cell anti-tumor responses. A better understanding of these molecular and cellular mechanisms is expected to enable the

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development of therapies to modulate the immune response for the benefit of the cancer patients.

Although most researchers agree that the malignant cells trigger the events that ultimately lead to T cell dysfunction, the intermediaries in this process vary, including regulatory T cells, plasmacytoid dendritic cells, suppressor macrophages, and more recently myeloid-derived suppressor cells (MDSC). The latter subpopulation of immune cells has recently become the focus of much research because of novel observations demonstrating their ability to regulate T cell responses, among others, by controlling the availability of the amino-acid L-Arginine (L-Arg). In this review, we will discuss some of the most recent concepts of how MDSC metabolizing L-Arg regulate T cell function in disease, and suggest possible therapeutic applications to inhibit their suppressive activity.

### 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 (Miescher et al., 1986; Miescher et al., 1988; Whiteside et al., 1988; Whiteside and Rabinowich, 1998). Initial explanations included the development of “blocking antibodies”, the production of suppressor factors and the generation of suppressor macrophages (Hellstrom et al., 1971; Hellstrom, Hellstrom, and Nelson, 1983; Varesio et al., 1979). However, the significance of these findings on the progression of the disease was unknown. Although cancer patients generally do not develop the characteristic opportunistic infections seen in patients immunosuppressed by high doses of chemotherapy, they show impaired T cell responses against bacterial and/or chemical antigens (Miescher et al., 1986; Miescher et al., 1988; Whiteside et al., 1988; Whiteside and Rabinowich, 1998).

This suggests that tumors have the ability to suppress T responses. In accordance, several animal tumor models and many clinical trials demonstrated that immunotherapy in both mice and patients with advanced tumors failed to achieve a therapeutic response as a result of the loss of T cell responses (Reviewed in Gattinoni et al., 2006; Sharma et al., 2011). In addition, several vaccine trials demonstrated the progression of tumors in spite of a robust T cell response (Stevenson, 2005). The development of cellular and molecular models leading to T cell anergy provided important insights on how cancer (and chronic inflammatory diseases) could selectively induce T cell dysfunction (Rabinovich, Gabrilovich, and Sotomayor, 2007).

This provided the basis for the discovery of several mechanisms including the role of immunoregulatory molecules in antigen presenting cells (APCs) such as B7.1, B7.2, B7-H1 and B7-H4 (Dong et al., 1999; Klausner, Lippincott-Schwartz, and Bonifacio, 1990; Kryczek et al., 2006b; Kryczek et al., 2006a), the development of regulatory T cells (McHugh et al., 2001; McHugh and Shevach, 2002), and the tumor-induced accumulation of MDSC (Bronte and Zanovello, 2005; Gabrilovich, 2004; Pekarek et al., 1995; Seung et al., 1995). Although most models are in agreement that tumor cells are the initiators of the suppressor phenomenon, they also conclude that APCs, in the form of macrophages, dendritic cells, and MDSC, play a central role in directly inducing T cell anergy (McHugh et al., 2001; McHugh and Shevach, 2002).

In fact, Gabrilovich et al. (1998); Zarour et al. (1996); and Dolcetti et al. (2010) demonstrated that 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 immature myeloid cells that induce T cell dysfunction. These myeloid cells blocked T cell responses by producing IL-10, TGF- $\beta$  and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (Young, Newby, and

Wepsic, 1987). Furthermore, an increased accumulation of these immature myeloid cells was found in patients with head and neck, breast, and lung cancer (Almand et al., 2000; Almand et al., 2001).

Intensive research has also focused in determining mechanisms by which T cells become suppressed in tumor-bearing hosts. Results showed that T cells from cancer patients and tumor bearing mice had multiple, but discrete changes in the expression of signal transduction molecules, including a decreased expression of the T cell receptor  $\zeta$  chain (CD3 $\zeta$ ), a diminished tyrosine kinases p56<sup>lck</sup>, p59<sup>fyn</sup>, and an inability to upregulate Jak-3 and to translocate NF $\kappa$ Bp65, all of which resulted in a diminished *in vitro* T cell response (Ghosh et al., 1994; Li et al., 1994; Mizoguchi et al., 1992).

These alterations were accompanied by a diminished ability to mobilize Ca<sup>++</sup> and a decreased tyrosine phosphorylation (Mizoguchi et al., 1992), providing a possible molecular explanation for the T cell dysfunction in cancer. These initial findings in mice were confirmed in patients with renal cell carcinoma, melanoma, Hodgkin's disease, ovarian cancer, colon carcinoma, and cervical cancer among others (Finke et al., 1993; Kono et al., 1996a; Zea et al., 1995). Preliminary studies also showed that patients with melanoma or head and neck tumors had a decreased survival time if expressing low levels of CD3 $\zeta$  (Kuss et al., 1999; Zea et al., 1995). The absence of a mechanism to explain these changes and the apparent lack of specificity of these alterations created some initial controversy around these observations.

Later, Otsuji et al. (1996) and Kono et al. (Kono et al., 1996a; Kono et al., 1996b) demonstrated that the co-incubation of activated murine peritoneal macrophages with T cells induced the loss of CD3 $\zeta$  chain. This phenomenon could be blocked by oxygen radical scavengers and was therefore thought to be mediated by hydrogen peroxide (Corsi et al., 1998). Similarly, Schmielau et al described an increased number of activated neutrophils in peripheral blood of patients with pancreatic and breast cancer who also had a diminished expression of CD3 $\zeta$  chain (Schmielau and Finn, 2001). In addition, Baniyash and colleagues proposed that chronic stimulation of T cells by specific antigens led to the decreased expression of CD3 $\zeta$  chain leading to the induction of anergy (Bronstein-Sitton et al., 2003). However, none of these models fully reproduced the multiple alterations found in T cells of cancer patients.

### Metabolism of L-Arg by Myeloid Cells

L-Arg is the substrate for four enzymes that exist as multiple isoforms: nitric oxide synthases (NOS1, NOS2, and NOS3), arginases (Arginase I and II), Arginine: glycine amidinotransferase (AGAT), and L-Arg decarboxylase (ADC) (Figure 1). Dietary L-Arg is taken up by intestinal epithelial cells and traverses the plasma membrane via the y<sup>+</sup> system of cationic amino acid transporters (CAT) (Closs et al., 2004). Inside the cell, L-Arg is metabolized by NOS enzymes to produce citrulline and nitric oxide, the latter of which plays an important role in cytotoxic mechanisms and vasodilatation (Amber et al., 1991; Hibbs, Jr., Taintor, and Vavrin, 1987). 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 the second an important mechanism for detoxification of protein degradation (Morris, Jr., 2002). ADC converts L-Arg to agmatine, which in turn is converted to putrescine and urea by agmatinase. ADC and AGAT appear to be less involved in the immune response. Mammalian ADC is highly expressed in brain (Iyo et al., 2006; Zhu et al., 2004), while AGAT is expressed in brain and heart (Cullen et al., 2006; Item et al., 2001).

The expression of arginase I and NOS2 in murine macrophages is differentially regulated by Th1 and Th2 cytokines (Hesse et al., 2001; Munder et al., 1999). Stimulation of murine macrophages with IFN- $\gamma$  up-regulates NOS2 exclusively, while IL-4, IL-10 and IL-13 induce arginase I (Munder, Eichmann, and Modolell, 1998; Rutschman et al., 2001) (Figure 1). The mitochondrial isoform arginase II is not significantly modulated by Th1 or Th2 cytokines (Rodriguez et al., 2003). The inhibition of arginase I leads to an increased NOS2 expression and consequently promotes NO production (Chicoine et al., 2004). Conversely, upregulation of arginase I inhibits NOS activity and contributes to the pathophysiology of several disease processes, including vascular dysfunction and asthma (Zhang et al., 2004).

The mechanism of inhibition of NOS2 expression by arginase I appears to be mediated by L-Arg depletion, which blocks the translation of NOS2 (Lee et al., 2003). In addition, low levels of nitric oxide induce nitrosylation of cysteine residues of arginase I, which increases the biological activity of arginase I, further reducing L-Arg (Santhanam et al., 2007).

Activation of murine macrophages with Th1 or Th2 cytokines also has different effects on the extra-cellular levels of L-Arg. Peritoneal macrophages stimulated with IL-4 plus IL-13 increase the expression of Arginase I and cationic amino acid transporter **2B** (CAT-2B), which results in a rapid increase in the uptake of extra-cellular L-Arg with the consequent reduction of L-Arg in the microenvironment. In contrast, macrophages stimulated with IFN- $\gamma$  preferentially express NOS2, do not increase CAT-2B, and do not deplete L-Arg from the microenvironment (Rodriguez et al., 2003).

Recent data from the arginase I and arginase II knockout mice confirm that only arginase I is able to deplete serum levels of L-Arg (Deignan et al., 2006; Iyer et al., 2002). Similarly, in vitro co-culture experiments showed that only macrophages producing arginase I, but not those expressing NOS2, caused the prolonged loss of CD3 $\zeta$  and inhibited T cell proliferation. The addition of arginase inhibitors or exogenous L-Arg reversed the CD3 $\zeta$  loss and reestablished T cell proliferation (Rodriguez et al., 2003). These results were confirmed with macrophages from arginase I conditional knockout mice (Unpublished findings).

### Effects of L-Arg Starvation in T cells

The association of L-Arg metabolism and T cell responses was established through experiments showing that the thymic involution and decrease in T cells seen in mice undergoing extensive surgery was prevented by the injection of LArg (1). Our initial experiments demonstrated that culturing T cells in tissue culture medium with L-Arg levels <50  $\mu$ M resulted in a significant decrease in cell proliferation (2). In addition, T cells activated in a L-Arg free environment developed all the alterations previously described in tumor bearing mice and cancer patients, i.e., the decreased expression of CD3 $\zeta$ , an inability to upregulate Jak-3, and a decreased translocation of NFkB-p65 (Zea et al., 2004).

Although the initial results suggested that the low T cell proliferation was the result of a decreased CD3 $\zeta$  expression, we found that T cells cultured in the absence of L-Arg had an increased production of IL-2 and high expression of early activation markers CD25, CD69, CD122, and CD132. In addition, T cells activated with PMA, which bypasses the T cell receptor, also failed to proliferate (3), confirming that the suppressive effect induced by the absence of L-Arg was not caused by a decrease in the T cell receptor signaling.

Later, Rodriguez et al. showed that activated T cells cultured in the absence of L-Arg were arrested in the G<sub>0</sub>-G<sub>1</sub> phase of the cell cycle, while cells cultured with L-Arg progressed into the S and G<sub>2</sub>-M phases (Rodriguez, Quiceno, and Ochoa, 2007). Association of D-type cyclins (Cyclin D1, D2 and D3) and cyclin-dependent kinase 4 (cdk4) and cdk6 regulate the

progression of T cells through early G<sub>1</sub> and later into the S phase of the cell cycle (Kato, 1997). Our results showed that T cells cultured in the absence of L-Arg were unable to up-regulate cyclin D3 and cdk4, but increased cdk6 expression (Rodriguez, Quiceno, and Ochoa, 2007). In addition, silencing of cyclin D3 in T cells induced a similar inhibition of proliferation as that seen in the absence of L-Arg. Interestingly, LArg starvation impaired the expression of cyclin D3 and cdk4 in T cells through a decreased mRNA stability and diminished translational rate (Rodriguez et al., 2010; Rodriguez, Quiceno, and Ochoa, 2007).

Previous results have suggested the mechanisms by which amino acid starvation impairs global translation. Accumulation of empty aminoacyl tRNAs caused by amino acid starvation, activates GCN2 kinase which phosphorylates the translation initiation factor eIF2 $\alpha$ . The phosphorylated form of eIF2 $\alpha$  binds with high affinity to eIF2B, blocking its ability to exchange GDP for GTP, which inhibits the binding of the eIF2 complex to methionine aminoacyl tRNA. This results in a decreased initiation of global protein synthesis.

Our recent results suggest that T cells cultured in the absence of L-Arg display a decrease in global translation, which associates with higher levels of phospho-eIF2 $\alpha$  (Rodriguez et al., 2010) (Figure 2). In addition, the global decrease in translation impairs the expression of RNA-binding protein HuR, thereby inhibiting mRNA stability of mRNA containing AUUA rich elements (Rodriguez et al., 2010). However, it is still unknown whether phospho-eIF2 $\alpha$  is the only mediator of the arrest in translation induced by the absence of L-Arg. Furthermore, T cells from GCN2 knockout mice do not show an arrest in cell cycle or a decrease in proliferation when cultured in medium without L-Arg, suggesting the potential role of GCN2 on the alterations induced by the absence of L-Arg (Rodriguez, Quiceno, and Ochoa, 2007).

### Arginase I Expression in MDSC Infiltrating Tumors

Several tumor lines including non-small lung carcinoma and breast carcinoma have been shown to express arginase (Chang, Liao, and Kuo, 2001; Singh et al., 2000; Suer et al., 1999). This has been thought to be a mechanism for the production of polyamines needed to sustain the rapid proliferation of tumor cells. Results from our laboratory suggest instead that arginase I is preferentially expressed in MDSC infiltrating tumors, which inhibits T cell function and represents a possible mechanism of tumor evasion (Rodriguez et al., 2004). Although the majority of the studies suggested that MDSC suppress T cell function, few reports initially showed the absence of this effect. These discrepancies were the result of MDSC isolated from different tissues (lymph node vs spleen vs tumor) or even in the differential tumor model. In addition, it is also accepted that MDSC initially accumulate to fight tumors, but are changed in their phenotype as tumor progresses and chronic inflammation establishes. Therefore, timing also plays a major role.

Two important subsets of MDSC have been reported based on their expression of CD11b, LY6G and LY6C. Granulocytic MDSC have a CD11b<sup>+</sup> LY6G<sup>+</sup> LY6C<sup>low</sup> phenotype, whereas monocytic MDSC are CD11b<sup>+</sup> LY6G<sup>-</sup> LY6C<sup>high</sup> (Youn et al., 2008). Although these populations have been identified, it is still unclear the biology and the mechanisms of the accumulation of these cells in disease. So far, monocytic MDSC appear to be precursors of macrophages and dendritic cells, whereas granulocytic MDSC are formed by mature and immature polymorphonuclear cells (Gabrilovich and Nagaraj, 2009). Granulocytic and monocytic populations of immature myeloid cells (iMC) with a phenotype similar to MDSC are present in the bone marrow of healthy mice (Bronte et al., 2000; Kusmartsev et al., 2004; Sinha, Clements, and Ostrand-Rosenberg, 2005a). However, recent publications suggest that



MDSC although morphologically similar to iMC, display a high T cell suppressive capability, whereas iMC lacks it (Solito et al., 2011; Youn et al., 2012).

Depletion of both MDSC subpopulation using anti-GR-1 antibodies induced an anti-tumor effect mediated by CD8<sup>+</sup> T cells (Holda, Maier, and Claman, 1985; Pekarek et al., 1995; Seung et al., 1995). Furthermore, we and others previously reported the existence of CD11b<sup>+</sup> LY6G<sup>-</sup> LY6C<sup>-</sup> MDSC in tumors, which have a similar phenotype that of alternatively activated macrophages (Dolcetti et al., 2010; Rodriguez et al., 2004). Even though there are variations in cell morphology and maturation markers between different tumor models and between murine and human tumors, these myeloid cells suppress T cell function. In addition, accumulating literature now describes the role of MDSC in angiogenesis and in the promotion of the pre metastatic niche (Granot et al., 2011; Toh et al., 2011).

Granulocytic MDSCs are the major source of arginase I in tumor bearing hosts (Rodriguez et al., 2004), whereas tumor-associated monocytic MDSC metabolize L-Arg through NOS2 (Youn et al., 2008).

Our data suggests that the primary mechanism by which MDSC induce T cell tolerance is by depleting extra cellular L-Arg through Arginase I (Rodriguez et al., 2005; Rodriguez et al., 2003; Rodriguez et al., 2004) (Figure 3). The addition of arginase I inhibitors Nor-NOHA or NOHA *in vitro*, or its injection into tumor-bearing mice prevents the loss of T cell function and results in an immune-mediated anti-tumor response, which inhibits tumor growth in a dose-dependent manner. The inhibition in tumor growth caused by Nor NOHA, does not happen in tumor-bearing *scid* mice, strongly suggesting that the anti-tumor effect caused arginase I inhibition is dependent on lymphocyte function (Rodriguez et al., 2004).

MDSC may also cause T cell tolerance through cell to cell contact. This mechanism appears to require the co-expression of arginase I and NOS2 (Bronte and Zanovello, 2005). The addition of NOS2 and arginase inhibitors to co-cultures of MDSC and activated T cells completely reestablishes T cell function (Bronte et al., 2003). It is possible that this phenomenon is in part mediated by the production of peroxynitrites. Under limiting amounts of L-Arg, NOS2 produces peroxynitrites (ONOO<sup>-</sup>), a highly reactive oxidizing agent that nitrates proteins and induces T cell apoptosis (Kusmartsev and Gabrilovich, 2005). This appears to affect the conformational flexibility of the T cell antigen receptor and its interaction with MHC by inducing nitration of T cell antigen receptor proteins in CD8<sup>+</sup> cells.

Thus, MDSC directly disrupt the binding of specific peptides on MHC to CD8<sup>+</sup> T cells (Lu et al., 2011; Nagaraj et al., 2007). In addition, peroxynitrites induce apoptosis in activated T cells (Kasic et al., 2011). MDSC co-expressing arginase I and NOS2 primarily impair CD8<sup>+</sup> T cell function (Kusmartsev and Gabrilovich, 2005; Rodriguez et al., 2004; Sinha, Clements, and Ostrand-Rosenberg, 2005a; Van Ginderachter et al., 2006) by blocking their ability to secrete IFN- $\gamma$  when stimulated with specific antigens (Gabrilovich et al., 2001; Kusmartsev et al., 2004), and inducing apoptosis (Kusmartsev and Gabrilovich, 2005). This suppression requires the production of IL-13 and IFN- $\gamma$  (Gallina et al., 2006; Sinha, Clements, and Ostrand-Rosenberg, 2005b; Sinha, Clements, and Ostrand-Rosenberg, 2005a) and signaling through STAT1 transcription factor (Kusmartsev and Gabrilovich, 2005). In addition, MDSC have been shown to produce high levels of stem cell factor (SCF) when stimulated by IFN $\gamma$  and IL10 (Huang et al., 2006). Blocking of SCF signaling in MDSC significantly impairs their ability to generate regulatory T cells (Pan et al., 2008), preventing a fundamental mechanism by which MDSC induce T cell suppression.

## MDSC in Human Tumors

Human MDSC phenotypes vary significantly ranging from immature myeloid cells (Sica and Bronte, 2007; Solito et al., 2011) to activated granulocytes (Rodriguez et al., 2009). They have been reported to express a wide range of markers including CD11b, CD14, CD15, CD33, VEGFR and CD66b (reviewed in (Greten, Manns, and Korangy, 2011)). A retrospective study of patients with metastatic renal cell carcinoma (RCC) demonstrated a 6–10 fold increase in arginase activity in the peripheral blood mononuclear cells (PBMC), as compared to normal controls (Zea et al., 2005). Separation of the different subpopulations in the PBMC of these patients demonstrated that the major source for arginase were granulocytes, which separated with the PBMC when centrifuged over ficoll-hypaque (Rodriguez et al., 2009). In addition, T cell proliferation and IFN- $\gamma$  production in vitro was re-established after the depletion of MDSC.

In the same patient population, there was an inverse statistical correlation between arginase activity and MDSC numbers with the expression of CD3 $\zeta$  chain in T cells (Figure 3). A similar sub-population of activated granulocytes had previously been described by Schmielau and Finn (Schmielau and Finn, 2001) in patients with pancreatic cancer, where they demonstrated a correlation between the presence of activated granulocytes and alterations in T cells such as reduced CD3 $\zeta$  expression and decreased cytokine production (Schmielau and Finn, 2001). Furthermore, results from clinical trials using IL-2 in patients with renal cell carcinoma and melanoma have shown a clear association between poor clinical responses and high numbers of granulocytes in peripheral blood (Rodgers, Rees, and Hancock, 1994).

Although human MDSC express high levels of arginase I, this does not appear to be upregulated by cytokines or other signals, suggesting the possibility of a constitutively expression of arginase I in human MDSC. Furthermore, human MDSC do not uptake L-Arg. Instead, arginase I stored in primary (Munder et al., 2005) or gelatinase granules (Jacobsen et al., 2007) is released to the microenvironment inducing a significant decrease in L-Arg levels, which impairs T cell function and CD3 $\zeta$  chain expression (Kropf et al., 2007; Munder et al., 2006; Zea et al., 2005) (Figure 3). In fact, the release of arginase I into the sera of renal cell carcinoma patients induced a decrease in plasma of L-Arg levels to  $<50\mu\text{M}$  and an increase in ornithine levels demonstrating that arginase I not only had a metabolic effect (L-Arg depletion), but also a negative effect on the T cell response (Zea et al., 2005).

## Regulation and Activation of MDSC in Cancer

Different cytokines participate in the recruitment of MDSC from the bone marrow, including VEGF and GM-CSF (Dolcetti et al., 2010). In addition, serum levels of VEGF directly correlated with numbers of MDSC in the blood and spleen (Ohm and Carbone, 2001) and have been associated with poor prognosis in cancer patients. Tumor derived VEGF has been previously associated with an arrest in dendritic cells maturation (Gabilovich et al., 1998; Oyama et al., 1998) through the inhibition in Nf- $\kappa\beta$  signaling. Treatment of MDSC with all-*trans* retinoic acid appears to counter this inhibition and promotes MDSC differentiation into mature APC (Kusmartsev et al., 2003). 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 (Bronte et al., 2000).

Similar effects on MDSC have been suggested with other growth factors including Fms-like tyrosine kinase 3 (Flt3) ligand (Solheim et al., 2007), stem cell factor (SCF) (Pan et al., 2008) and S100 calcium binding protein A9 (S100A9) (Cheng et al., 2008). Furthermore, blocking of MDSC accumulation in tumor-bearing hosts with antibodies against Gr-1, CD11b, and colony stimulating factor 1 (CSF1), using the inhibitor of CSF1 receptor

(CSFR1/c-kit), sunitinib, or using the anti-metabolites gemcitabine (GEM) and 5-fluorouracil (5-FU) has shown to partially restore T cell function in tumor bearing hosts (Gabrilovich and Nagaraj, 2009; Ko et al., 2010; Kusmartsev et al., 2008; Le et al., 2009; Mundy-Bosse et al., 2011; Pan et al., 2008; Toh et al., 2011; Vincent et al., 2010). However, it remains unclear the effects of the mentioned chemotherapy agents in the accumulation of specific sub populations of MDSC. I.e. Sunitinib blocks the proliferation of monocytic MDSC, while impairing granulocytic MDSC survival (Ko et al., 2010). This important question is being tested in ongoing research in several labs.

We used the 3LL murine lung carcinoma model to further determine what factors might be inducing the production of arginase I in the MDSC infiltrating tumors. MDSC isolated from 3LL tumors and placed in culture in regular medium, lose arginase I expression within 24 hours. However, if freshly isolated MDSC were co-cultured in transwells with 3LL cells or with 3LL supernatants they maintained arginase I expression, suggesting that the induction of arginase I was caused by soluble factor(s) produced by tumor cells (Rodriguez et al., 2005).

Cytokines such as IL-4, IL-13, TGF- $\beta$  and others were not detected in the supernatants of the 3LL single cell suspensions. Instead, we detected a very high expression of the inducible cyclooxygenase-2 (COX-2) and an increased production of prostanoids including PGE<sub>2</sub>. The addition of COX-2 inhibitors or silencing of COX-2 in 3LL cells, completely blocked their ability to induce arginase I in MDSC (Rodriguez et al., 2005).

The effect of PGE<sub>2</sub> on MDSC was dependent on the expression of the E-prostanoid receptor (EP4) on MDSC and was associated with an increased cAMP levels (Rodriguez et al., 2005). Consequently, treatment of tumor bearing mice with COX-2 inhibitor sc-58125 decreased the expression of arginase I in MDSC infiltrating the tumor and induced an immune-mediated anti-tumor effect (Rodriguez et al., 2005). Similar results have been reported in mice bearing the 4T1 breast carcinoma where the treatment with the COX-2 inhibitor SC-58236 reduced the accumulation of MDSC in the spleen in an EP2 dependant manner (Sinha et al., 2007).

In addition celecoxib, a selective COX-2 inhibitor was able to inhibit the induction of colon tumors in Swiss mice treated with 1,2-dimethylhydrazine diHCl-(1,2-DMH) (Talmadge et al., 2007). Other factors may also play a role in the induction of arginase in MDSC including hypoxia-inducible factor 1 (HIF-1) and HIF-2 (Reviewed in (Semenza, 2003)), IL-4, IL-13 and IFN  $\gamma$  (Gallina et al., 2006).

Furthermore, although the mechanisms controlling the induction of arginase I in MDSC have been partially identified in mice, the factors mediating the activation of MDSC in cancer patients remain unclear. A recent publication by Roton et al. (2009) suggests the possible role of tumor derived IL-8 on the release of arginase from human MDSC. In addition, Solito et al. (2011) showed that a combination of G-CSF and GM-CSF induce a heterogeneous promyelocyte population with characteristics of MDSC. Future clinical approaches either inhibiting the factors leading to the accumulation of human MDSC or depleting MDSC can be predicted. This will create an opportunity for combination with different chemotherapy agents, cancer vaccines, and/or other forms of immunotherapy.

## MDSC: LESSONS FROM OTHER DISEASES AND FUTURE APPLICATIONS

The advent of immunotherapy of cancer made apparent that in spite of powerful biological agents that could prime tumor specific T cells, tumors had sophisticated mechanisms to escape the immune response, including MDSC. MDSC however are not unique to cancer. Trauma patients and patients with chronic infections including active pulmonary



tuberculosis also have increased numbers of MDSC expressing arginase I that inhibit T cell function. This data suggests that MDSC 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. A demonstration of this mechanism was described in the late 1980s by Albina et al. (1989) studying the healing of surgical wounds.

They described that the tissue surrounding a surgical wound was initially infiltrated by cells expressing iNOS, which would most likely eliminate offending 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. 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 difficult its surgical excision. 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 MDSC. In contrast, malignant tumors do not stop growing and destroying tissue (would not “heal”) promoting instead a chronic inflammatory process mediated by MDSC. 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, it is our hypothesis that tumors “hijack” a normal healing process making it instead a vicious cycle that results in the inhibition of a potentially protective T cell anti-tumor 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 new therapeutic approaches to interrupt this dysfunctional response.

In summary, our understanding of the role that MDSC play in the progression of tumors has significantly increased during the last 10 years. Multiple approaches have been taken to block MDSC suppression including the use of all-transretinoic acid (Kusmartsev et al., 2003), inhibiting nitric oxide function with nitro-aspirin (De et al., 2005), inhibiting phosphodiesterase-5 (Serafini et al., 2006), blocking arginase activity with specific arginase inhibitors (Rodriguez et al., 2004), blocking of MDSC accumulation using antibodies against Gr-1, CD11b, and CSF, or using chemotherapy agents sunitinib, GEM, or 5-FU (Gabrilovich and Nagaraj, 2009; Ko et al., 2010; Kusmartsev et al., 2008; Le et al., 2009; Mundy-Bosse et al., 2011; Pan et al., 2008; Toh et al., 2011; Vincent et al., 2010). It is likely that the appropriate combination of inhibitors blocking MDSC function and stimuli protecting T cells may overcome this powerful tumor-derived mechanism that impairs the promise of cancer immunotherapy.

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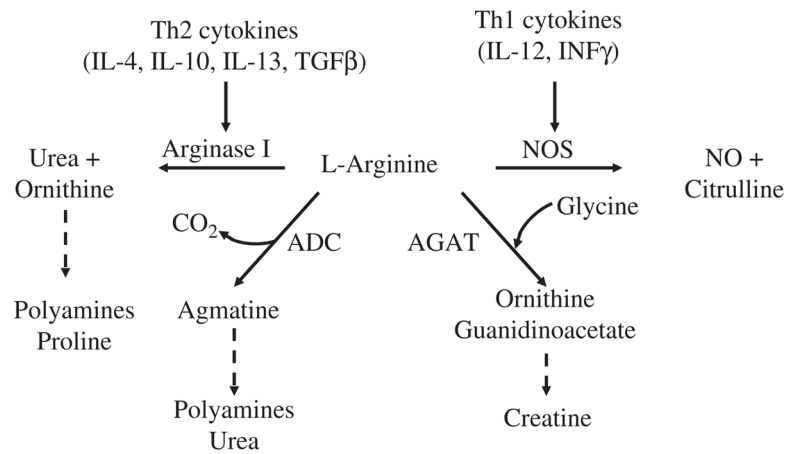
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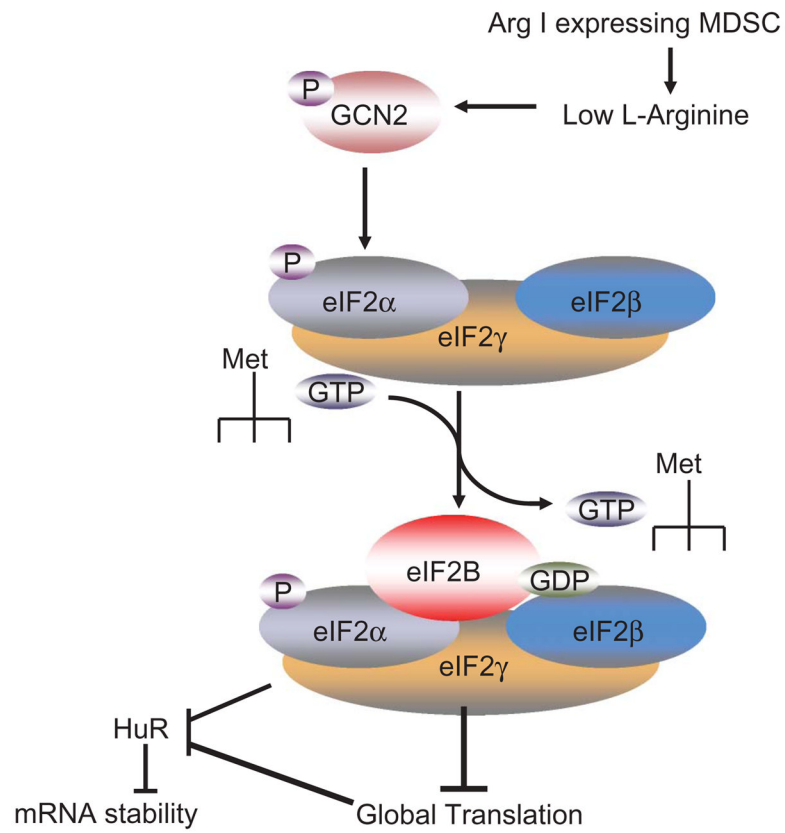
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**Figure 1. L-Arg metabolism**

For the sake of simplicity, emphasis is on enzymes that directly metabolize L-Arg. This diagram should not be interpreted to indicate that all of these enzymes are expressed simultaneously in any given cell type. Th2 cytokines lead to the increase expression of arginase I, while Th1 cytokines increase inducible NOS. ADC, Arginine decarboxylase; AGAT, arginine: glycine amidinotransferase; NO, nitric oxide; NOS, nitric oxide synthase.

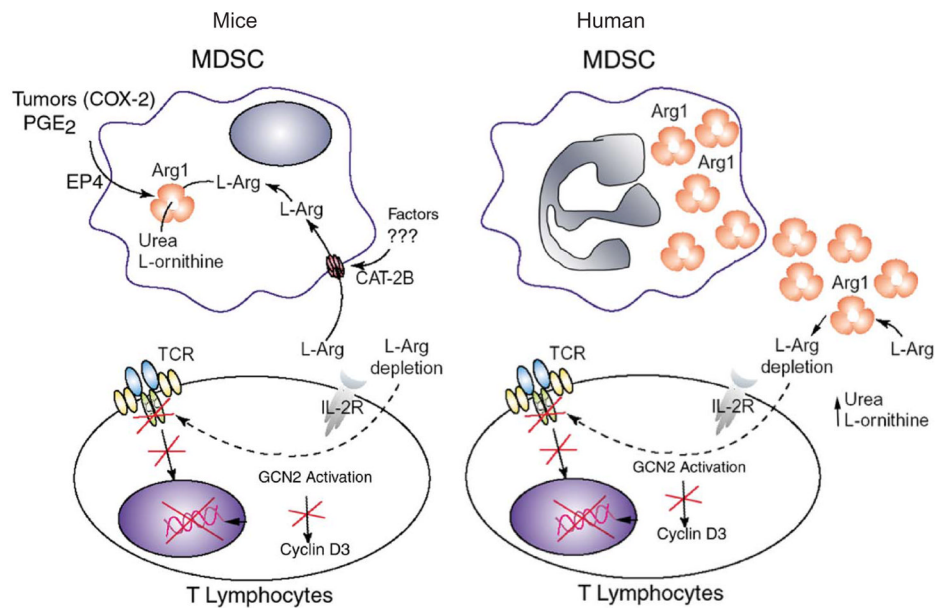
T cells exposed to low levels of L-Arginine



**Figure 2. Control of T cell translation by MDSC**

Metabolism of L-arginine by MDSC leads to a decrease in the extra cellular availability of arginine, which in T cells activates GCN2 kinase, thereby leading to the phosphorylation of eIF2a. This leads to a global decrease in translation and to the low expression of mRNA stabilizing protein HuR.





### Figure 3. T cell dysfunction induced by arginase I

Tumor cells expressing COX-2 and releasing PGE2 induces the expression of arginase I and CAT-2B in MDSC. This leads to a reduction of extra cellular levels of L-Arg, which finally activates GCN2 and blocks the expression of multiple genes including CD3 z, cyclin D3 and cdk4. A similar reduction occurs in patients with cancer, but through the release of arginase into the extracellular environment.