[Frontiers in Bioscience E4, 1237-1245, January 1, 2012]

Natural suppressor cells; past, present and future

Parvin Forghani[1], M.R. Khorramizadeh[1], Edmund K. Waller[2]

[1]School of Public Health, Dept of Medical Biotechnology, School of Advanced Medical Technologies Tehran University of Medical Sciences, Tehran, Iran, [2]Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA

TABLE OF CONTENTS

1. ABSTRACT

Myeloid Derived Suppressor Cells (MDSCs) are a mixed group of bone marrow–derived myeloid cells containing macrophages, granulocytes, immature DCs and early myeloid precursors that have immune suppressive activity (1). MDSCs infiltrate the BM, spleen and peripheral blood of tumors-bearing experimental animals and are found in the blood of cancer patients as a result of tumor-induced alterations in myelopoiesis. Evidence from murine model systems indicated that myeloid-derived cells with suppressor activity also accumulate in non-tumor bearing hosts in response to infection, chemotherapy (2), stress (3), and immune senescence(4). MDSCs are considered key negative regulators of immune responses. Their association with tumor-associated immune defects make MDSCs an attractive target for therapeutic intervention in cancer.

2. HISTORY OF NATURAL SUPPRESSOR CELLS

The history of "natural suppressor cells" (NS cells) began with a report that graded numbers of radiosensitive/ non-adherent normal bone marrow cells inhibited antibody response of spleen cells in vitro (5,6). Further experiments showed that normal bone marrow cells suppress the generation of cytotoxic cells in MLR (7). The natural suppressor cells were characterized by absence of detectable B or T cell markers, thymic independency and heat-sensitive suppressive activity (8), However, fractionation of bone marrow cells by velocity sedimentation identified a population of medium-to-large FC receptor-positive cells that lacked T cell, B cell, or macrophage markers(9,10). Their suppressive effect in MLR could not be abrogated by the Natural Killer(NK) -cell-depleting anti-asialo GM1 antibody leading to the suggestion that the NS cells were T cell precursors (11) or

immature myeloid cells (6). A short time after the discovery of NS cells in murine BM, a group of radiosensitive, Ia negative cells were identified in human bone marrow as analogs of murine natural regulatory cells. These human suppressor cells shared the capability to suppress antibody response in-vitro (12). These findings raised the possibility that these cells had a role in the physiological regulation of immune responses.

While most of the work in the field has focused on natural suppressor cells in the myeloid lineage, another line of investigation has characterized a T cell suppressor population. Natural suppressor cells were noted in the spleen of neonatal mice shortly after birth (13), in normal adult mice after total lymphoid irradiation, and in murine allograft BM recipients that develop Graft versus host disease(GVHD) and following treatment with cyclophosphamide and vaccines (14,15). This population has been described as a lymphoid surface marker null, non-adherent, non-phagocytic cell (15). Thymic independent, $CD4^-$ $CD8^-$ TCR alpha/ beta positive lymphoid suppressor cells have been identified in murine (16) and human BM (17) and represent a very low number of blood T-cells in mice and humans. These rare T-cells are considered apart of the immuno suppressive network that is able to suppress MLR and GVHD (17, 18), but these cells have not been implicated in tumor-associated immune suppression.

There have been a multitude of descriptions for tumor-associated myeloid-derived suppressor cells in mice and man. The first evidence that myeloid natural suppressor in BM could regulate tumor growth was provided by showing inhibition of lymphoma, myeloma cell proliferation cell lines in vitro (19). Ten years later the potent immuno-suppressive effects of bone marrow derived NS cells in lung tumor-bearing mice was recognized (20) and myeloid-derived suppressor cells were described in the blood of patients with head and neck carcinoma (21). A number of reports described elevated number of immature myeloid cells (IMC) in tumor specimens and in secondary lymphoid organs. These myeloid cells were thought to be generated during tumor-derived inflammation and were believed to be responsible for maintaining an immuno-suppressed state(22). The term "inhibitory macrophages" (iMacs) has also been used to describe a heterogeneous population of mature and immature myeloid progenitor cells that were responsible for suppressing CD8+ T-lymphocyte function (25). Later the term "Myeloid-Derived Suppressor Cells" (MDSC) was recommended as an alternative to iMac or immature myeloid cells (26), and as an alternative to the term “myeloid suppressor cells (MSC)" that was also a common abbreviation for mesenchymal stromal cell (27). The term MDSC was preferred because it described both the origin and the function of these cells in mediating tumor - associated immune suppression(28). At the time the term “MDSC” was developed, it was believed that theses cells did not have an immuno-suppressive effect in tumor-free mice. The term “MDSC” was used to distinguish these tumor-related immuno-suppressive cells from immature myeloid cells (IMC) in non-tumor bearing mice that were felt to lack immunosuppressive activity (1).

During last decade, while CD11b+ Gr1+ MDSCs have been identified as the predominant suppressor of anti tumor immune response by cancer immunologists and biologists, the significance and biological role of these cells in non-tumor bearing animals has been largely ignored. In this review, we aim to take a comparative look at the natural suppressor cells present in the mice and human.

3. FEATURES OF MDSCS

3.1. Cell surface markers

MDSCs in various murine tumor models typically express the αM integrin CD11b and myeloid lineage differentiation antigen “Granulocyte Receptor 1” (GR-1), that are related to monocytic and granulocytic lineages, respectively (1, 24) and express variable levels of Ly6-G, Ly6-C on their surface (23, 24). Depending on the on particular tumor microenvironments from which they are isolated, they may express additional markers. Recently CD49d (an integrin α-subunit) expression has been used in combination with CD11b to identify CD11b/CD49d a phenotypically and functionally distinct MDSC subtype (30). Other cell surface markers such as IL-4R alpha, F4/80, CD80, CD31, and CD115 have been used to delineate myeloid cell subsets with immunosuppressive activity (25, 31, 32). Based on theses cell surface markers and nuclear morphology MDSCs have been classified in to two major subsets: Mononuclear cells that are $CD11b^+Ly6G^{+/-}Ly6C^{hi}CD49d^+$ and are considered "monocytic” and $CD11b^+$ $Ly6G^+Ly6C^{Low/-}$ CD49d- cells with multilobed nuclei that have a “granulocytic” phenotype (30, 33). Several lines of evidence indicate that the phenotype of these cells in tumor-bearing mice is not identical to the immature myeloid cells in non-tumor bearing animals. Immature myeloid cells in the spleen of non-tumor bearing mice are typically monocytic with a CD11b+Ly6C+Ly6G– phenotype and express higher levels of CD71, CD115, and CD80 than MDSCs derived from tumors (32). While these molecules may also be associated with MDSCs, their expression does not appear to be necessary for the immuno-suppressive activity of tumor-derived MDSC.

The phenotype of human MDSCs is not as well described as that of murine MDSC. A few published studies have investigated the clinical significance of human MDSCs in patients with a variety of different cancers. Human MDSCs lacked surface markers of mature lymphoid or monocytic cells including: CD3, CD19, CD57, CD14, HLA DR but expressed the myeloid markers CD33 and CD11b and CD15 (33). A new population of MDSC with the CD14+HLA-DR–/low immunophenotype has been identified in peripheral blood mononuclear cells of patients with prostate cancer and melanoma, suggesting that different human tumors may induce different subtypes of MDSC (34, 35). However the functional attributes of the different subsets of human MDSCs remain to be defined.

3.2. Suppressive properties

The defining quality of MDSCs is that they suppress adaptive and innate immune responses. Multiple

reports have shown that a group of Gr-1+CD11b+ myeloid cells accumulate in murine tumors during cancer progression and that CD11b+ Gr-1+ cells isolated from tumors can inhibit antitumor T-cell responses (1, 2, 24). This immunosuppressive activity of tumor-associated murine MDSC on different T cell subsets varies according to culture conditions. Some studies have shown immediate inhibition of MHC class I-restricted CD8+ T cells by Gr-1+ MDSC (36, 37), whereas CD4+ T cell inhibition has been observed after several day incubation (38). While MDSCs can affect T-cell responses as immature cells in the peripheral lymphoid organs, their differentiation into more mature cell types can be regulated by the tumor microenvironment. The immuno-suppressive activity of MDSC is quite distinct from the immunological activity of classically activated macrophages (M1), generated following stimulation with IFN-gamma and LPS. In contrast to MDSC, M1 macrophages exhibit potent microbicidal properties and promote IL-12-mediated Th1 responses. The immuno-suppressive activity of immature CD11b+ MDSC is also distinct from alternatively activated (M2) macrophages that have a mature phenotype and can be further subdivided in M2a macrophages (following exposure to IL-4 or IL-13), M2b macrophages (stimulated by immune complexes in combination with (IL-1β or LPS) and M2c macrophages (stimulated by IL-10, TGF beta or glucocorticoids) (39). The immuno-suppressive activity of MDSC appears to depend upon the maintenance of an immature state, as MDSC that differentiate may lose their suppressive effect even though they continue to express the myeloid marker CD11b.

MDSCs have been recognized as critical mediators of immune evasion in some but not all human solid tumors (1, 12, 40). When MDSC have been identified in human tumors, the tumor type and tumor stage affect the distribution and number of MDSC subpopulations. Expression of the granulocytic marker CD15 has been used to divide human MDSC into two subsets: a CD15+ subset in patients with kidney cancer, able to suppress T-cell function through an arginase and/or reactive oxygen species-dependent mechanism and a CD15− subset of MDSCs that suppress T-cell function in patients with carcinoma through a yet to be defined mechanism (34, 41, 42). The distinct immunosuppressive mechanisms of different human and mouse MDSC populations that have been described suggests that differences in the maturation state of MDSC may correspond to specific immunosuppressive pathways, and provides a possible explanation for that distinct in vitro differentiation potential of phenotypically defined MDSC subsets (43, 44).

3.3. Molecular Mechanisms of suppressor activity of MDSCs

The fundamental quality of Gr-1+/CD11b+ MDSC detected in all murine tumors is that they impair T and NK cell activation. MDSCs reduce antitumor immunity through a diverse array of mechanisms that impact the activation as well as effector functions of both innate and adaptive immunity. The mechanism for immuno-suppressor activity appear to vary across different MDSC sub fractions (44). The various mechanisms for the immunosuppressive activity of these cells are reviewed below in order of descending frequency of reports that support a particular mechanism for the immuno-suppressive activity of MDSCs.

3.3.1. NO – dependent mechanisms

A significant portion of the ability of MDSC to suppress T cells has been attributed to the generation of Peroxynitrite (ONOO-.). Peroxynitrite is generated by arginase (Arg) and inducible nitric oxide synthetase (iNOS) enzymatic activities that have been characterized in MDSC in mouse and human models (36, 42, 43). Nitrite is a stable end product of NO, a powerful oxidant that produces by iNOS during the conversion of L-arginine to L-citrolin. iNOS induced by a number of cytokines including IFN gamma and TNF in macrophage and myeloid cells. iNOS inhibits T cell activation and proliferation by: blocking of tyrosine phosphorylation of Janus kinase 3, STAT5 transcription factor, and inhibition of MHC class II gene expression and the induction of apoptotic death (45, 46).

3.3.1.1. iNOS & Arginase (ARG1) up regulation

There is a strong association between suppressive activity of MDSCs with the metabolism of L-arginine in murine and human tumors models. MDSCs express high levels of cytoplasmic Argenase1 that is induced by Th2 cytokines. Arginase hydrolyses the amino acid l-arginine to ornithine and urea. Arginase synergizes with iNOS to give rise to peroxynitrites that cause the apoptosis of T lymphocytes by inhibiting protein tyrosine phosphorylation via nitration of tyrosine residue (47, 37). There is also evidence for cooperation between IFN-gamma and IL-13 cytokines, leading to the activation of Arg1 and Nos2 enzymes that mediate TCR zeta-chain down-regulation, a common finding in most cancer patients (48, 49). Many tumor cells also over expressed and secreted COX-2, a key enzyme in the biosynthesis of PGE2 that leads to enhanced arginase production. COX-2 inhibitors have been shown slow tumor growth in mouse models (50). However fundamental differences have been reported between mice and humans for the role of iNOS and Arg1. For example Arg-1 is expressed in murine but not in human M2 macrophages. These discrepancies may reflect fundamental differences in immune regulatory pathway between human and mice or the incomplete homology of particular MDSC subsets across the two species (51, 52).

3.3.1.2. ROS-mediated cell killing

Another mechanism that has been associated with the immuno-suppressive activity of MDSC is the generation of Reactive Oxygen Species (ROS). ROS are very short-lived substances that are present in higher concentrations in MDSCs generated from tumors. ROS are induced following up-regulation of NADPH oxidase (NOX2) subunits (p47phox and gp91phox) by STAT3 and IFN-gamma signaling. The presence of ROS- dependent MDSC activity in IFN- γ receptor- deficient mice suggests that IFN-gamma is dispensable for ROS production in MDSC (53, 54). As previously mentioned, there are difference between murine MDSCs with respect to the role ROS in immune suppression. While monocytic MDSC have activated STAT1 which up regulates iNOS and

generates NO, Granulocytic MDSC suppress through high levels of reactive oxygen species that are induced following increased activation of STAT3 and NADPH which induce apoptosis of T cells, and drive nitrosylation T cell receptors rendering them unable to bind peptide/MHC complexes (54, 44).

3.3.2.1. Blocking of NK cytotoxicity

MDSC are also expanded during acute inflammation and the initiation of innnate immunity. NK cells, a key component of the innate immune system, can modulate the activity of MDSC. MDSC, in turn, can also suppress the antigen non-specific cytotoxicity of NK cells and the role of NK cells in initiating adaptive immune responses through IFN-gamma, synthesis (required for Th1 differentiation of naïve T cells). While multiple pathways are involved in MDSC–mediated T cell suppression, the mechanism of NK cell inhibition by MDSC is not thoroughly understood. There are controversies in the literature regarding crosstalk between MDSC and NK cells. TGF beta1 is an important suppressive cytokine produced by MDSCs in tumor-bearing mice that impairs splenic & hepatic NK cell activity through down regulation of NKG2D (55, 56). On the contrary, other studies showed that NKG2D ligand RAE-1 is exclusively expressed on Gr-1+CD11b+F4/80+ MDSCs from lymphoma and melanoma tumor-bearing mice. This interaction activates NK cells and simulates their production of IFN gamma (57). However, recently published evidence still supports the role of MDSC-mediated NK cell suppression (58).

3.3.2.2. Development of Foxp3+ T regs

MDSC have also been shown to support the generation of Foxp3+ regulatory T cells (Tregs) thereby limiting T-cell activation. T regs are part of the immunosuppressive network and have been targeted for therapeutic interventions (59). Several reports suggest that MDSCs play an important role in inducing T reg from naïve T cells but not in the conversion of regulatory to FOXP3+ T cells from FOXP3− effectors T cells (60, 61). Induction of T reg by immature myeloid DCs has been identified both in vitro and in tumor-bearing mice. T reg induction by MDSC requires IFN-gamma and IL-10 but not IL-4 (61). Some studies have been identified also TGF beta as a requirement for induction of FOX-P3 T reg in vitro by MDSCs (62), suggesting that different MDSC subpopulations may activate Tregs through disparate mechanisms.

3.3.2.3. Skewing of macrophage activity

Several studies indicate that MDSC-induced immunosuppressive macrophages inhibit T cells (25, 26). In the tumor microenvironment MDSCs modulate tumor-associated macrophages (TAM) and induce immunosuppressive (M2) phenotype versus the (M1) phenotype that has anti tumor activity (63). In contrast to most immature myeloid cells that are matured by inflammatory stimuli and support the generation of effective Th1 cellular immune responses, following exposure to proinflamatory signals MDSCs maintain an immature phenotype and contribute to deviation of M1 macrophages towards M2 macrophages that are relatively tumor-promoting with increased production of IL-10 and down regulation of IL-12 (64). My regulating the generation of M2 macrophages in the tumor micro-environment MDSCs influence innate and adaptive immune responses that limit the generation of anti-cancer immunity.

3.3.2.4. Down regulation of L-selectin

L-selection (CD62L) expression is required for homing of naive T cells to peripheral lymph nodes and is down regulated by MDSC (65). CD4+ or CD8+ T cells co-cultured with MDSC had a L-selectin low phenotype, suggesting that MDSC directly down-regulate T cell expression of L-selectin. Also L-selectin-deficient mice have shown decreased numbers of T cells infiltrating tumors, supported the hypothesis MDSC regulate trafficking of naive T cells to tumors. Additional experiments showed MDSC cleave L-selectin due to constitutive expression of metalloproteinase ADAM17 that degrade L-selectin (66, 67). Therefore, MDSCs can reduce anti tumor immunity by interfering with the ability of naive T-cells to traffic to the sites of tumor-associated antigen presentation in lymph nodes, decreasing adaptive anti-tumor immune responses.

3.3.2.5. Cystine Sequestration

Recently the deprivation of necessary amino acid involved in T cell proliferation from the extracellular environment has been described as an additional MDSC-mediated suppressive mechanism. MDSCs deplete cystine and cysteine from extracellular fluid thereby inhibiting T-cell activation. Of note, the highest requirement for cysteine is when T cells are activated and proliferated following their interaction with antigen presenting cells (APC). However, during antigen presentation, T cells and APC are in very close proximity, so cysteine released by APC can be directly taken up by nearby T cells. Compared with monocytes MDSCs have higher concentration of intracellular stores of cystine. MDSCs in thetumor microenvironment directly compete with APC for cysteine uptake. Thus increased numbers MDSCs act as a cystine sink, and limit the activation and proliferation of T-cells (68).

In addition to the above-mentioned mechanisms cell-cell contact, and some unknown soluble molecules contribute to the immunosuppressive activity of MDSC (69). However these mechanisms are not restricted to cancer as inflammation, sepsis, autoimmune disease and transplantation induce MDSCs with similar immuno suppressive properties.

3.4. Activation & expansion of mdscs

3.4.1. Cytokine-dependence of MDSC

The accumulation of MDSC appears to be a dominant and common process for immune suppression in rodents as well as in humans with cancer. Several findings have been suggested that MDSCs are induced by inflammation and inhibit immuno-surveillance (43, 48). Mice with cancer have increased numbers of MDSC in their BM, blood, spleen and liver compared with non-tumor-bearing mice, while increased numbers of MDSC

have been noted in the blood of patients with cancer, including melanoma (70) breast (71), hepatocellular carcinoma (72) and renal cell carcinoma (73). A variety of tumor-derived factors such as GM-CSF, M-CSF, IL-3, IL-10, TGF beta, VEGF IL-6 and IL-1β have been implicated in the development and accumulation of MDSCs (1, 73). A common finding of many reports is that stem cell factor (SCF, ckit ligand) expressed by multiple human and murine tumor cell lines is a critical factor for MDSC accumulation (74). SiRNA-knockdown of SCF in breast, melanoma and colon cell lines have resulted in fewer infiltrating MDSC in tumor-bearing mice compared with mice bearing control mock-transduced tumors, and T cells recovered from mice treated with anti c-kit siRNA secreted higher levels of IFN-gamma and lower levels of the immuno-suppressive cytokines IL-10 and TGF beta (75). Granulocyte macrophage–colony-stimulating factor(GMCSF) is released by macrophages, T and NK cells and has been shown to elicit powerful immune responses *in vivo* where it has been used as a immune adjuvant in various murine models and in clinical vaccine trials. Several reports have highlighted a dual role of GM-CSF in MDSC physiology, as low dose of GM-CSF has different effects compared with high dose GM-CSF. Low dose GM-CSF has limited anti tumor efficacy and resulted in significant immunosuppression mediated by MDCSs (76).

3.4.2. Modulation of VEGF signaling by MDSC

Vascular endothelial growth factor(VEGF) regulates crosstalk between tumor cells and MDSCs. Neoangiogenesis is important in the expansion and mobilization of MDSCs and MDSCs directly promote tumor angiogenesis and tissue remodeling via production VEGF, TGF beta and Matrix Metalloproteinase - (MMP9)(77). A strong correlation between the number of MDSCs in highly vascular tumors such as breast, bladder and prostate carcinoma with the expression an array of angiogenesis modulating enzymes such as MMP-2, -7, -9 and -12 has been shown (78). Transgenic mice expressing HER-neu develop mammary carcinoma and the progression of these tumors is correlated with serum levels of VEGF (79). Increased levels VEGF are correlated with high numbers of immature dendritic cells in the peripheral blood of patients with gastric, lung and head and neck carcinoma (77).

3.4.3. Tumor-directed myeloid differentiation towards MDSC

Tumor associated cytokines and chemokines may also drive neutrophil differentiation towards MDSC and enhance MDSC homing to tumors. In addition of tumor, TGF-beta, produced by MDSCs in the tumor microenvironment, drives differentiation of tumor-associated neutrophils (TANs) toward the N2 pro tumorigenic phenotype (80). Several other chemo attractants have also been shown to be involved in the recruitment of myeloid cells by tumors. MDSCs express one or more chemokine or growth factor receptors including CCR2, CXCR4, CXCR2, CD117 and VEGFR1 (81, 82). Among there, CCR2 has been considered as a marker for MDSCs. Interaction of CCR2 positive MDSC with CCL2 provides an important signaling pathway for MDSC recruitment to the tumor microenvironment (83). There is also evidence that MDSCs migrate to tumors in response to cell damage, infection, or inflammatory mediators belonging to the S100 family of calcium binding proteins (S100A12, S100A8, and S100A9)(84). These proteins mediate accumulation of MDSC in the local inflammatory microenvironment of tumors through blocking the differentiation of myeloid precursors into differentiated DC chemoattracttion of MDSCs to tumor sites (NF-κB-dependent pathway) (1). Thus to overcome MDSC mediated immune suppression, it is crucial to identify tumor factors that are relevant across a variety of tumor models that may represent “drugable” targets for new pharmaceuticals designed to block for MDSC differentiation or accumulation in tumor-bearing mice and humans.

3.5. Perspective

While the focus of the MDSC field has been their role in the tumor microenvironment, it is becoming increasingly clear that similar myeloid cells in non-tumor-bearing animals play a significant role in regulating immune responses in the bone marrow microenvironment. It is clear that memory CD8+ T cells, senescent neutrophils, plasma cells and memory B cells recirculate from peripheral lymphatic tissues to the bone marrow, but that immune activation does not normally occur at this site. The BM has been identified as a reservoir of myeloid cells and plasma cells and the site for hematopoiesis. However, considering that natural regulatory cells within the bone marrow constitute the largest fraction of nucleated cells in this tissue, it is reasonable to posit that the BM is an immuno-privileged site that limits the initiation of antigen-specific immune responses. In this case, natural regulatory cells act as sensors of T cell activation and cooperate to inhibit further inflammatory activation pathways that might reaction interfere with normal hematopoiesis. Considering that “double-negative” T-cells in the bone marrow also have unique immuno-suppressive behavior, the presence of myeloid and lymphoid natural suppressor cells in the bone marrow may function to limit interactions of B cell with T cells crucial for germinal center constitution. The emerging appreciation of the immuno-suppressive role of MDSCs in non-tumor bearing animals situation will change the dogma concerning lack of suppressive effect of IMC in the bone marrow in the future. In addition, understanding the precise role of MDSCs in the bone marrow microenvironment and their interaction with resident T cells will be important in bone marrow transplantation (BMT) and/or BM- related diseases such as myelodysplastic syndrome (MDS).

4. SUMMARY

Natural suppressor cells were initially identified as null cells with suppressive activity that were not affected by monoclonal antibodies to T-cell antigens. Natural suppressor cells were distinct from NK cells and lymphoid precursors based on the patterns of cell surface molecules and their function. The term of MDSCs was developed to describe a heterogeneous monocytic-granulocytic population with immuno-suppressive activity that comprises the majority of cells in human and mouse

BM. Despite studies spanning two decades, their role of MDSC in anti-tumor immunity and the mechanisms involved in activation and accumulation of these cell in tumor bearing models and in steady state non tumor bearing animals remains only partially understood. The evolution of this field will include insights as to how the regulatory processes employed by MDSC that have been implicated in control of anti-tumor immunology apply to normal bone marrow physiology.

5. REFERENCES

1. DI Gabrilovich, S Nagaraj S: Myeloid - derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9,162-174 (2009)

2. CM Diaz-Montero, ML Salem, MI Nishimura, E Garrett-Mayer, DJ Cole, AJ Montero: Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 58, 49-59 (2009)

3. VP Makarenkova, V Bansal, BM Matta, LA Perez, JB Ochoa: CD11b+/Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. J Immunol176, 2085-94 (2006)

4. EY Enioutina, D Bareyan, RA Daynes: A role for immature myeloid cells in immune senescence. J Immunol 186, 697-707 (2011)

5- JA Bennett, VS Rao, MS Mitchell: Systemic bacillus Calmette-Guérin (BCG) activates natural suppressor cells. Proc Natl Acad Sci 75, 5142-4 (1978)

6. JS Corvese, EM Levy, M Bennett, SR Cooperband: Inhibition of an in vitro antibody response by a suppressor cell in normal bone marrow. Cell Immunol 49, 293-306 (1980)

7. S Muraoka S, RG Miller: Cells in bone marrow and in T cell colonies grown from bone marrow can suppress generation of cytotoxic T lymphocytes directed against their self antigens. J Exp Med 152, 54-71(1980)

8. AK Duwe, SK Singhal: The immunoregulatory role of bone marrow. II. Characterization of a suppressor cell inhibiting the in vitro antibody response. Cell Immunol 43, 372-81 (1979)

9. K Dorshkind, C Rosse: Physical, biologic, and phenotypic properties of natural regulatory cells in murine bone marrow. Am J Anat 164, 1-17 (1982)

10. RC McGarry, SK Singhal. The immunoregulatory role of bone marrow. III. Further characterization of the suppressor cell and its mode of action. Immunology 46, 387-94 (1982)

11. MJ Dauphinée, N Talal : Failure of NZB spleen to respond to prethymic bone marrow suppressor cells. J Immunol 122, 936-41 (1979)

12. MA Bains, RC McGarry, SK Singhal: Regulatory cells in human bone marrow: suppression of an in vitro primary antibody response. *Cell Immunol* 74, 150-61 (1982)

13. G Rodriguez, G Andersson, H Wigzell, AB Peck: Non-T cell nature of the naturally occurring, spleen-associated suppressor cells present in the newborn mouse. *Eur J Immunol*, 9,737-46 (1979)

14. Strober S: Natural suppressor (NS) cells, neonatal tolerance, and total lymphoid irradiation: exploring obscure relationships. *Annu Rev Immunol* 2,219-37(1984)

15.A Oseroff, S Okada, S Strober: Natural suppressor (NS) cells found in the spleen of neonatal mice and adult mice given total lymphoid irradiation (TLI) express the null surface phenotype. *J Immunol* 132,101-10 (1984)

16. V Palathumpat, S Dejbakhsh-Jones, B Holm,H Wang, O Liang, S Strober: Studies of CD4- CD8- alpha beta bone marrow T cells with suppressor activity. *J Immunol* 48, 373-80 (1992)

17. IG Schmidt-Wolf, S Dejbakhsh-Jones, N Ginzton, P Greenberg, S Strober: T-cell subsets and suppressor cells in human bone marrow. *Blood* 80, 3242-50 (1992)

18.V Palathumpat, S Dejbakhsh-Jones,S Holm B, S Strober: Different subsets of T cells in the adult mouse bone marrow and spleen induce or suppress acute graft-versus-host disease. *J Immunol* 149, 808-17 (1992)

19. K Sugiura, M Inaba, H Ogata, R Yasumuzu, EE Sardiña et al: Inhibition of tumor cell proliferation by natural suppressor cells present in murine bone marrow. *Cancer Res* 50, 2582-6 (1990)

20. MR Young, MA Wright: Myelopoiesis-associated Immune Suppressor Cells in Mice Bearing Metastatic Lewis Lung Carcinoma Tumors: γ Interferon plus Tumor Necrosis Factor α Synergistically Reduces Immune Suppressor and Tumor Growth-promoting Activities of Bone Marrow Cells and Diminishes Tumor Recurrence and Metastasis. *Cancer Res* 52, 6335-40 (1992)

21. MR Young, MA Wright, Y Lozano, JP Matthews, J Benefield, MM Prechel : Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. *Int J Cancer* 67, 333-8 (1996)

22. B Almand, JI Clark, E Nikitina, J Van Beynen, NR English, SC Knight, DP Carbone, DI Gabrilovich: Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol* 166, 678-89 (2001)

23. AD Billiau, S Fevery, O Rutgeerts, W Landuyt, M Waer: Transient expansion of Mac1+Ly6-G+Ly6-C+ early myeloid cells with suppressor activity in spleens of murine radiation marrow chimeras: possible implications for the graft-versus-host and graft-versus-leukemia reactivity of donor lymphocyte infusions. *Blood* 102,740-8 (2003)

24. V Bronte, E Apolloni, A Cabrelle, R Ronca, P Serafini, P Zamboni, NP Restifo, P Zanovello: Identification of a CD11b (+)/Gr-1 (+)/CD31 (+) myeloid progenitor capable of activating or suppressing CD8 (+) T cells. *Blood* 96, 3838-3846 (2000)

25. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, Basso G, F Brombacher, Borrello I, P Zanovello, S Bicciato, V Bronte: Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T. *J Clin Invest* 116, 2777-90 (2006)

22. B Almand, JI Clark, E Nikitina, J Van Beynen, NR English, SC Knight, DP Carbone, DI Gabrilovich: Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol* 166, 678-89 (2001)

23. AD Billiau, S Fevery, O Rutgeerts, W Landuyt, M Waer: Transient expansion of Mac1+Ly6-G+Ly6-C+ early myeloid cells with suppressor activity in spleens of murine radiation marrow chimeras: possible implications for the graft-versus-host and graft-versus-leukemia reactivity of donor lymphocyte infusions. *Blood* 102,740-8 (2003)

24. V Bronte, E Apolloni, A Cabrelle, R Ronca, P Serafini, P Zamboni, NP Restifo, P Zanovello. Identification of a CD11b (+)/Gr-1 (+)/CD31 (+) myeloid progenitor capable of activating or suppressing CD8 (+) T cells. *Blood* 96, 3838-3846 (2000)

25. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, Basso G, F Brombacher, Borrello I, P Zanovello, S Bicciato, V Bronte. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T. *J Clin Invest* 116, 2777-90 (2006)

26. Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S: Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J Immunol* 176, 284-90 (2006)

27. P Sinha, VK Clements, S Ostrand-Rosenberg: Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease: *J Immunol* 174, 636-645 (2005)

28. DI Gabrilovich , V Bronte , SH Chen , MP Colombo , A Ochoa , S Ostrand-Rosenberg , H: The terminology issue for myeloid-derived suppressor cells. *Cancer Res* 67, 425 (2007)

29. G Krystal , L Sly , F Antignano , V Ho , J Ruschmann , Hamilton M: Re: the terminology issue for myeloid-derived suppressor cells. *Cancer Res* 67, 3986 (2007)

30. LA Haile, J Gamrekelashvili, MP Manns, F Korangy, TF Greten. CD49d is a new marker for distinct myeloid-derived suppressor cell subpopulations in mice. *J Immunol* 185, 203-10 (2010)

31. V Bronte, P Serafini, A Mazzoni, DM Segal, P Zanovello: L-arginine metabolism in myeloid cells controls T-lymphocyte functions. *Trends Immunol* 24,302-306 (2003).

32. DI Gabrilovich, MP Velders, EM Sotomayor, WM Kast: Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol* 166, 5398-406 (2001)

33. I Poschke, D Mougiakakos, J Hansson, GV Masucci, Kiessling R. Immature immunosuppressive CD14+HLA-DR-/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. *Cancer Res* 70, 4335-45(2010)

34. S Vuk-Pavlović, PA Bulur, Y Lin, R Qin, CL Szumlanski, X Zhao, AB Dietz. Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate cancer. *Prostate* 70, 443-55 (2010)

35. P Filipazzi, R Valenti, V Huber, L Pilla,P Canese, M Iero,C Castelli, L Mariani, G Parmiani, L Rivoltini. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. J Clin Oncol 25, 2546- 53 (2007)

36. DI Gabrilovich , MP Velders , EM Sotomayor , WM Kast: Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol* 166, 5398-406 (2001)

37. V Bronte, P Serafini, C De Santo, I Marigo, V Tosello, A Mazzoni, Segal DM, Staib C, M Lowel, G Sutter, MP Colombo, P Zanovello: IL-4-Induced Arginase 1 Suppresses Alloreactive T Cells in Tumor-bearing mice. *J Immunol* 170, 270-278 (2003)

38. LS Cauley, EE Miller, M Yen, SL Swain Cauley . Super antigen-induced CD4 T cell tolerance mediated by myeloid cells and IFN-γ. J Immunol 165, 6056–66 (2000)

39. FO Martinez, A Sica, A Mantovani, M Locati. Macrophage activation and polarization. Front Biosci 13:453-61(2008)

40. M Terabe, S Matsui, JM Park, et al: Transforming growth factor-β production and myeloid cells are an effector mechanism through CD1d-restricted T cells block cytotoxic T-lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. *J Exp Med* 198, 1741–52 (2003)

41. HJ Bühring, S Treml, F Cerabona , P de Zwart, L Kanz, M Sobiesiak : Phenotypic characterization of distinct human bone marrow-derived MSC subsets. *Ann N Y Acad Sci* 1176, 124-34 (2009)

42. PC Rodriguez, MS Ernstoff, C Hernandez, M Atkins, J Zabaleta, R Sierra, AC Ochoa: Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma

are a subpopulation of activated granulocytes. *Cancer Res* 69, 1553-60 (2009)

43. S Ostrand-Rosenberg, P Sinha: Myeloid-Derived Suppressor Cells: Linking Inflammation and Cancer. J Immunol 182, 4499 -4506 (2009)

44. K Movahedi , M Guilliams , J Van den Bossche , R Van den Bergh , C Gysemans , A Beschin , P De Baetselier , JA Van Ginderachter : Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood 111: 4233-44 (2008)

45. A Mazzoni, V Bronte, A Visintin, JH Spitzer, E Apolloni, P Serafini, P Zanovello, DM Segal: Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. J Immunol 15,168(2): 689-95 (2002)

46. CS Lin, HC Ho, KC Chen, G Lin, L Nunes, TF Lue: Intracavernosal injection of vascular endothelial growth factor induces nitric oxide synthase isoforms. BJU Int 89, 955-60 (2002)

47. V Bronte, P Serafini, A Mazzoni, DM Segal, P Zanovello: L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol 24,302-306 (2003)

48. AV Ezernitchi, I Vaknin, L Cohen-Daniel, O Levy, E Manaster, Halabi A, Pikarsky E, L Shapira, M Baniyash: TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. J Immunol 177, 4763-72 (2006)

49. M Baniyash: TCR zeta-chain down regulation: curtailing an excessive inflammatory immune response. Nat Rev Immunol 4, 675-87 (2004)

50. C Murdoch, M Muthana, SB Coffelt, CE Lewis: The role of myeloid cells in the promotion of tumor angiogenesis. Nat Rev Cancer 8,618-31 (2008)

51. G Raes, R Van den Bergh, P De Baetselier, GH Ghassabeh, C Scotton, M Locati, A Mantovani, S Sozzani: Arginase-1 and Ym1 are markers for murine, but not human, alternatively activated myeloid cells. J Immunol 174, 6561 (2005)

52. M Munder, F Mollinedo, J Calafat, J Canchado, Gil-Lamaignere C, Fuentes JM, C Luckner, G Doschko, G Soler, K Eichmann, FM Müller, AD Ho, M Goerner, M Modolell: Arginase-I is constitutively expressed in human granulocytes and participate in fungicidal activity. Blood 105, 2549-2556 (2005)

53. S Kusmartsev, Y Nefedova, D Yoder, DI Gabrilovich: 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 (2004)

54. S Kusmartsev, D I Gabrilovich: Inhibition of myeloid cell differentiation in cancer: the role of reactive oxygen species. J Leukocyte Biol 74,186-196 (2003)

55. C Liu, S Yu, J Kappes, J Wang, WE Grizzle, KR Zinn, HG Zhang: Expansion of spleen myeloid suppressor cells represses NK cell cytotoxicity in tumor-bearing host. Blood 109, 4336–4342 (2007)

56. H Li, Y Han, Q Guo, M Zhang, X Cao: Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. J Immunol 182, 240-9 (2009)

57. N Nausch, IE Galani, E Schlecker, A Cerwenka: Mononuclear myeloid-derived "suppressor" cells express RAE-1 and activate natural killer cells. Blood 112, 4080-4089 (2008)

58. M Moshe Elkabets , VSG Ribeiro, GA Dinarello, S Ostrand-Rosenberg , J.P Di San, RN Apte, CAJ Vosshenrich: IL-1β regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. Euro J Immunol 40, 3347–3357(2010)

59. PY Pan, J Ozao, Z Zhou, SH Chen: Advancements in immune tolerance. Adv Drug Deliv Rev 60, 91-105(2008)

60. KP MacDonald, V Rowe, AD Clouston, JK Welply, RD Kuns, JL Ferrara, GR Thomas : Cytokine expanded myeloid precursors function as regulatory antigen-presenting cells and promote tolerance through IL-10-producing regulatory T cells. J Immunol 174, 1841-50 (2005)

61. P Serafini, S Mgebroff, K Noonan, I Borrello: Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer Res 68, 5439-49 (2008)

62. P Alard, SL Clark, MM Kosiewicz: Mechanisms of tolerance induced by TGF beta-treated APC: CD4 regulatory T cells prevent the induction of the immune response possibly through a mechanism involving TGF beta. Eur J Immunol 34, 1021-30 (2004)

63. P Allavena, A Sica, C Garlanda, A Mantovani: The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. Immunol Rev 222, 155–161(2008)

64. N Umemura, M Saio T Suwa, Y Kitoh, J Bai, K Nonaka, GF Ouyang, Okada M, M Balazs, R Adany, T Shibata, T Takami: Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. J Leukoc Biol, 83, 1136-44 (2008)

65. EM Hanson , VK Clements , P Sinha , D Ikovitch , Ostrand-Rosenberg S. : Myeloid-derived suppressor cells

down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J Immunol* 183, 937-44 (2009)

66. S Ostrand-Rosenberg: Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. *Cancer Immunol Immunother* 59, 1593-600 (2010)

67. MK Srivastava, P Sinha, VK Clements, P Rodriguez, S Ostrand-Rosenberg. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res* 70, 68–77 (2010)

68. S Rössner, C Voigtländer, C Wiethe, J Hänig, C Seifarth, MB Lutz. Myeloid dendritic cell precursors generated from bone marrow suppress T cell responses via cell contact and nitric oxide production in vitro. *Eur. J. Immunol* 35, 3533-3544 (2005)

69. P Filipazzi, R Valenti, V Huber, L Pilla , P Canese, M Iero , C Castelli , L Mariani , G Parmiani, L Rivoltini: Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J Clin Oncol* 25, 2546-53 (2007)

70. TF Greten, MP Manns, F Korangy. Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol* (2011)

71. B Hoechst, LA Ormandy , M Ballmaier , F Lehner , C Krüger , MP Manns , TF Greten, F Korangy: A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4 (+) CD25 (+) Foxp3 (+) T cells. *Gastroenterology* 135, 234-43 (2008)

72. S Kusmartsev, Z Su , A Heiser, J Dannull E Eruslanov, H Kübler , D Yancey , P Dahm , J Vieweg : Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. *Clin Cancer Res* 14, 8270-8 (2008)

73. JE Talmadge: Pathways mediating the expansion and immunosuppressive activity of myeloid-derived suppressor cells and their relevance to cancer therapy. *Clin Cancer Res* 13, 5243-8 (2007)

74. PY Pan, GX Wang, B Yin, J Ozao, T Ku, CM Divino, SH Chen: Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. *Blood* 111, 219-28 (2008)

75. P Serafini, R Carbley, KA Noonan, G Tan, V Bronte, I Borrello: High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res* 64, 6337-43 (2004)

76. J Marx: Cancer immunology: Cancer's bulwark against immune attack: MDS cells. *Science* 319, 154-6 (2008)

77. DI Gabrilovich, T Ishida, T Oyama: Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 92, 4150–66 (1998)

78. Y Sawanobori, S Ueha, M Kurachi, T Shimaoka, JE Talmadge, J Abe, Y Shono, M Kitabatake, K Kakimi, N Mukaida, K Matsushima: Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. *Blood* 111, 5457-5466 (2008)

79. C Melani, C Chiodoni, G Forni, MP Colombo: Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. *Blood* 102, 2138-45 (2003)

80. ZG Fridlender, J Sun, S Kim, V Kapoor, G Cheng, L Ling, Worthen GS, SM Albelda: Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 16, 183-94 (2009)

81. JE Talmadge, M Donkor, E Scholar: Inflammatory cell infiltration of tumors: Jekyll or Hyde. *Cancer Metastasis Rev* 26,373-400 (2007)

82. ZG Fridlender, V Kapoor, G Buchlis, G Cheng, J Sun, LC Wang, S Singhal, LA Snyder, SM Albelda: CCL2 Blockade Inhibits Lung Cancer Tumor Growth by Altering Macrophage Phenotype and Activating CD8+ Cells. *Am J Respir* Cell Mol *Biol* 44, 230-7 (2010)

83. P Sinha, C Okoro, D Foell, HH Freeze, S Ostrand-Rosenberg, G Srikrishna. Proinflammatory S100 Proteins Regulate the Accumulation of Myeloid-Derived Suppressor Cells. *J Immunol* 181, 4666 -4675 (2008)

84. P Cheng, CA Corzo, N Luetteke, B Yu, S Nagaraj, MM Bui, M Ortiz, W Nacken, C Sorg, T Vogl, J Roth, DI Gabrilovich: Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *JEM* 10, 2235-2249 (2008)

Abbreviations: MDSC: myeloid derived suppressor cell; BM: bone Marrow; NS: natural suppressor cells, IMC: immature myeloid cells; MSC: myeloid suppressive cells; APC: antigen presenting cells ; TReg: T regulatory; SCF, stem cell factor; TAN: tumor associated neutrophils; NOX2: NADPH oxidase; iNOS: inducible nitric oxide synthetas; ROS: Reactive Oxygen Species; TAM: tumor-associated macrophages, VEGF: vascular endothelial growth factor , GM-CSF: Granulocyte macrophage–colony-stimulating factor; GVHD: Graft versus host disease; SCF: stem cell factor; VEGF: vascular endothelial growth factor ; GR-1: Granulocyte Receptor 1

Key Words: Natural suppressor cell, Myeloid derived suppressor cells, bone marrow, Mouse, Human, Review

Send correspondence to: Edmund K. Waller, Winship Cancer institute, Emory University Medical School, Atlanta, 1365 Clifton Road NE, Room B5119GA30322, Tel: 404-727-4995, Fax: 404-778-5530, E-mail: ewaller@emory.edu