Regulatory Myeloid Suppressor Cells in Health and Disease

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Introduction

Research in recent years has brought a wealth of information about the important role of immune suppressive myeloid cells in cancer and other diseases. There is growing evidence suggesting that the expansion of these regulatory cells may represent a common response to all forms of inflammation. Several clinical trials target these cells with the goal of improving the immune response in cancer. However, the field as well as the term “regulatory myeloid cells” is highly controversial, and many issues remain unresolved. These issues were discussed at the international meeting in Clearwater, Florida held from March 8 through 11, 2009. Approximately 240 researchers working on myeloid-derived suppressor cells (MDSC), regulatory (suppressive) dendritic cells, as well as suppressive macrophages in cancer, infectious diseases, sepsis, trauma, and autoimmune diseases convened. Participants from different fields of research were provided with the opportunity to assess the data about expansion, accumulation, differentiation, regulation, and function of immune suppressive myeloid cells within the context of immunology of cancer and other diseases.

Myelopoiesis and the Origins of Suppressive Myeloid Cells

Chemokines, soluble and cell bound ligands, and micro RNAs regulate the differentiation of hematopoietic cells (HPC) into mature myeloid cells. Dr. Hal E. Broxmeyer (Indiana University School of Medicine, Indianapolis, IN) began this session with a talk on regulation of the function of HPC. Mobilization, homing, and engraftment of HPC were shown to be highly dependent on the expression of several cytokines, such as stromal cell-derived factor 1 (SDF-1), granulocyte macrophage colony-stimulating factors (GM-CSF), G-CSF, interleukin-3 (IL-3), and erythropoietin (Epo). Soluble and cell-bound dipeptidylpeptidase IV/CDC26 were able to cleave the amino-terminal dipeptide from cytokines and thus negatively regulate their chemotactic activity. Specifically, inhibition of cell-surface CD26 peptidase on HPC was shown to enhance their in vitro chemotaxis directed by SDF-1 and CXCL12, and in vivo homing and engraftment. Thus, inhibition of CD26 might enhance the efficacy of HPC transplantation. The myelosuppressive capability of CCL2, CCL3, CCL19, CCL20, CXCL4, CXCL5, CXCL8, CXCL9, and CXCL11 when used in combination exhibited a synergistic suppression in vivo. Furthermore, it was shown that the myelosuppressive ability of these chemokines, as well as of iron-binding proteins, was dependent on class II transactivator-regulated expression of major histocompatibility complex class II antigen, and CD26 cleavage enhanced the inhibitory activity of these myelosuppressive chemokines.

Dr. Fernando Camargo (Whitehead Institute for Biomedical Research, Cambridge, MA) investigated the role played by microRNAs in regulating myeloid gene expression using loss-of-function approaches and proteomic analysis. Micro-RNA-223 (miR-223) expression was restricted to the myeloid compartment. Proliferation and differentiation of this compartment was negatively regulated by miR-223. miR-223 mutant mice exhibited neutrophil hyperactivation resulting in inflammatory lung pathology and exaggerated tissue destruction after an endotoxin challenge. It was shown that MeF2c, a transcription factor promoting myeloid progenitor proliferation was a target of miR-223. In fact, MeF2C deficiency in miR-223 mutant mice suppressed progenitor expansion and partially corrected the neutrophil hypermaturation and hyperactivation phenotype. This suggested that miR-223 could control granulocyte production and the inflammatory response.

Interaction between progenitor cells and the stroma mediated by soluble and cell-bound factors greatly influences the potential of HPC to differentiate into dendritic cells (DC). Evidence linking Notch-1 and Wnt signaling and their role in DC differentiation and maturation were discussed. It was shown that activation of Notch signaling by Delta promotes DC differentiation, which was significantly impaired by a Notch specific inhibitor, GSI. This effect of the inhibitor was completely reversed by the activation of Wnt signaling, supporting the premise that Wnt signaling is a downstream event of Notch signaling (1).

MDSC in Trauma, Sepsis, and Burns

Dr. Cora K. Ogle (University of Cincinnati College of Medicine, Cincinnati, OH) showed that MDSC not only play a prominent role in the mechanisms of innate and adaptive immune response to cancer, but they have also been found to be accumulated in the course of some other pathologic processes such as thermal injury and sepsis. Granulocyte and macrophage subsets of immature myeloid cells (IMC) accumulate in spleen and blood, but not in the bone marrow (BM), of burn-injured mice, accompanied by an increase in the production of G-CSF. In contrast, scald injury increased the accumulation of a subset of monocytic inflammatory MDSC in BM, as well as in blood and spleen. Gemcitabine seemed to have an effect on MDSC accumulation in burn animal models, depleting subsets of immature polymorphonuclear neutrophils (PMN) and inflammatory monocytes, although sparing mature macrophages. In addition, isolated burn inflammatory monocytes suppressed lymphocyte proliferation and increased NO production. Gemcitabine treatment abolished burn injury-induced NO production, which correlated with decreased ability to suppress lymphocyte proliferation.
in murine model of thermal injury. MDSC in thermal injury constitute a major source of postburn inflammation.

In sepsis, expansion of HPC and IMC accounts for part of the supply of cellular effectors required to fight off infections. MDSC were dramatically elevated in BM and spleen and suppressed CD8+ T cells of septic mice (2). Mouse models of polymicrobial sepsis have gained substantial relevance as experimental approaches intended to elucidate mechanisms underlying the signaling circuits linked to this expansion. Particularly, CXCL12 and stem cell factor (SCF) had been implicated in differentiation and proliferation of common myeloid progenitors (CMP) and hematopoietic stem cell (HSC). Utilization of a CXCL12-blocking antibody prevented the accumulation of MDSC and myeloid progenitors in spleen of septic mice. Inhibition of CXCL12 during sepsis reduced their survival, suggesting a crucial role for CXCL12 in MDSC expansion during sepsis. Finally, the ability of anti-SCF antibody to abrogate the sepsis-induced accumulation of HSC and MDSC was discussed.

Systemic lupus erythematosus is characterized by excess type-I interferon (IFN-I) production, expression of IFN-stimulated genes, and production of auto-antibodies to DNA and ribonucleoproteins. Using 2,6,10,14-tetramethylpentadecane (TMPD) to induce a lupus-like disease in mice, Dr. Westley Reeves (University of Florida, Gainesville, FL) showed that disease progression was completely dependent on IFN-1 signaling. TMPD promotes IFN-1 production, recruitment of immature Ly6Chi monocytes, and autoantibody production. This recruitment was dependent on a Toll-like receptor 7 and myeloid differentiation factor 88-regulated pathway but independent of FcγR signaling.

Biological Role of Myeloid Cells in Cancer and Other Pathological Conditions

Immunosuppressive tumor-associated macrophages (TAMs) also play an important role in malignant progression. The nuclear factor κB (NF-κB) signaling pathway was reported to be involved in the maintenance of the immunosuppressive phenotype that characterizes TAMs. In a mouse model of ovarian cancer, the silencing of NF-κB signaling in macrophages resulted in a reduced ability to produce cytokines IL-10 and tumor necrosis factor-α (TNF-α) and an elevated production of IL-12. The increased production of IL-12 correlated with decreased tumor burden and increased the natural killer (NK) cell recruitment to the tumor site. It was also shown that signaling through the TNFα receptor in CD4+ T cells promoted tumor growth and recruitment of neutrophils to the tumor site. TNFα receptor activity in CD4+ T lymphocytes promoted their polarization to IL-17-producing cells. In addition, the cytokine IL-17 contributed to tumor burden and neutrophils recruitment.

In a model of skin melanoma presented by Dr. Viktor Umansky (German Cancer Center, Heidelberg, Germany) over-expression of receptor tyrosine kinase in melanocytes resulted in activation of the mitogen-activated protein kinase (MAPK) signaling pathway and development of spontaneous skin melanoma lesions, which eventually metastasized to various organs including lymph nodes, liver, lungs, and brain. It was shown that MDSC play a role in tumor rejection. Using a lymphoma model RMA-S, in which lymphoma cells did not express MHC-I molecule and NK cells were required for tumor rejection, a population of monocytic Gr-1+, CD11b+, F4/80+ with the potential to suppress T-cell responses was described by Dr. Adelheid Cervenka (German Cancer Center, Heidelberg, Germany). Interestingly, this Gr-1+, CD11b+, F4/80+ MDSC expressed the ligand RAE-1 for the NK cell-activating receptor NKG2D. MDSC did not prevent NK cells from destroying tumor cells in vitro. Contrarily, in the presence of MDSC, NK cells produced elevated levels of IFN-γ. In this model, depletion of MDSC enhanced the growth of RMA lymphoma in vivo.

A novel population of immuno-regulatory myeloid cells, referred to as regulatory macrophages (Mac-regs), which are present in mice under normal conditions, was introduced by Dr. Joseph Lustgarten (Mayo Clinic, Scottsdale, AZ). These CD11b+F4/80+ macrophages express the transcription factor Foxp3, which was previously associated with Tregs. Similar to Tregs, Foxp3 expressing CD11b+F4/80+ macrophages inhibited the proliferation of T cells, whereas Foxp3 negative macrophages did not. In addition to their ability to suppress T lymphocyte responses, it was suggested that Mac-regs were critical for the induction and maintenance of immune tolerance. Mac-regs were shown to support the induction of Tregs in response to antigenic stimulation.

Tumor antigen-specific T-cell tolerance is one of the major mechanisms of tumor escape. Dr. Srinivas Nagaraj (Moffitt Cancer Center, Tampa, FL) showed that MDSC suppression was not only restricted to a transgenic experimental model; it could also be observed in a nontransgenic experimental model expressing rat Neu. This finding was also confirmed in adoptive transfer experiments with CD8+ T cells expressing the dual TCR specific for LCMV glycoprotein peptide gp33-41 and OVA-derived H-2Kb-restricted peptide ova257-264. MDSC loaded with peptide specific for one TCR induced tolerance only against that specific epitope, but CD8+ T cells retained the ability to respond to the peptide specific for the other TCR expressed on the same cell.

Regulatory Myeloid Cells in Autoimmunity and Transplantation

Immature DCs are known to inhibit T effector cell function and promote the generation of Tregs. The characterization and generation of tolerogenic-immature DCs have thus led to several DC-based approaches to improve transplant tolerance. Dr. Angus Thomson (University of Pittsburgh School of Medicine, Pittsburgh, PA) showed that rapamycin-conditioned DCs represented immature-tolerogenic DCs that poorly stimulated allogeneic CD4+ T cells, induced antigen-specific Tregs, and promoted allograft survival.

Following the above discussion, Dr. Jacques Banchereau (Baylor Institute of Immunology Research, Waco, TX) presented data about the functions and localization of human skin myeloid DC subsets. Dermal CD14+ DCs were able to stimulate naïve CD4+ T cells into T helper cells that promoted isotype switching and conversion of naïve B cells to plasma cells. CD207+ epidermal Langerhans cells (LCs) promoted the differentiation of Th2 cytokine-secreting CD4+ T cells and efficiently primed CD8+ T cells. Finally, a third DC population, CD14+ CD207+ CD14a- dermal DCs could prime CD8+ T cells better than CD14+ but worse than LCs. These studies showed specialized DCs on the basis of their localization and differentiation.

SI2-domain containing 5’inositol phosphatase (SHIP), expressed by hematopoietic cells, has been shown to affect the repertoire of NK receptors in which its deficiency promoted an up-regulation of
specific inhibitory receptors. SHIP-deficient mice, capable of accepting fully mismatched allogeneic marrow grafts without graft-versus-host disease, had increased accumulation of MDSCs and Tregs (3).

Immune Suppressive Myeloid Cells in Cancer

TAMs, MDSC, and Tregs form a vicious network in suppressing the immune system in various pathological and cancerous conditions. It is known that TAMs are a prototypic M2 population that promotes angiogenesis and inflammation. Unraveling the cellular and molecular mechanism employed by TAMs would be crucial in targeting tumors (4). Because MDSC can suppress various immunological responses, their therapeutic use has the potential to increase the efficacy of BM and solid organ transplantation. Dr. Vincenzo Bronte (Istituto Oncologico Veneto, Padova, Italy) presented data to establish culture conditions to generate MDSC from BM cells (BM-MDSC). These cultured BM-MDSC suppressed antigen and nonantigen-specific CD8+ T cells and supported proliferation of Tregs. Furthermore, the use of BM-MDSC in vivo resulted in long term acceptance of allograft.

Vascular adhesion protein-1 (VAP-1), an endothelial cell-surface enzyme, is known to mediate lymphocytes and endothelial cell interaction (Kaisa Auvinen, National Public Health Institute, Turku, Finland). VAP-1 was also involved in the myeloid-cell recruitment into the inflamed area and to the tumor site. The percentage of MDSC in tumor was decreased in VAP-1 KO mice. These mice showed about 40% reduction of tumor volume and fewer CD31+ vessels in tumor.

Glucocorticoid treatment generated a specific monocyte phenotype with anti-inflammatory properties. It was shown that these regulatory monocytes are Gr-1+CD11b+ and express IL-4Ralpha chain (CD124), a signature molecule of tumor-induced MDSC, and suppress T cells in vivo (5).

Transforming growth factor β (TGF-β) is a very important mediator in tumor progression. Dr. Li Yang (National Institutes of Health, Bethesda, MD) presented that tumor-infiltrating MDSC exhibited a significantly elevated TGF-β signaling when compared with normal myeloid cells. They also produce a high level of multiple matrix metalloproteinases (MMP). These MDSC contributed to TGF-β-mediated metastasis through enhanced tumor cell invasion and metastasis. Moreover, mice with conditional knockout of TGF-βII receptor in MDSC showed a significantly attenuated 4T1 tumor growth compared with the control littermates.

Approaches to Therapeutic Correction of Myeloid Suppressor Cells

MDSC have been shown to substantially limit the therapeutic effect of cancer vaccination and immune therapy. Different strategies that target these cells were discussed at the meeting.

Phosphodiesterase-5 (PDE5) inhibitors (sildenafil) are agents currently in clinical use for nonmalignant conditions. Sildenafil treatment decreased the suppressive activity of MDSC by down-regulating arginase-1 (Arg-1) and inducible nitric oxide synthase-2 (NOS-2) expression. Sildenafil restored in vitro T-cell proliferation of peripheral blood mononuclear cells from multiple myeloma patients. By reverting MDSC suppression, sildenafil enhanced intratumoral T-cell infiltration and reduced tumor outgrowth in vivo (6). Cyclooxygenase 2 (COX-2) inhibitor (celecoxib) significantly reduced the number of splenic MDSC and also decreased mRNA levels of Arg-1 and NOS-2 in tumor-bearing mice. Similar to sildenafil, celecoxib delayed tumor induction and increased lymphocyte infiltration of tumors (7). Treatment with amino-biphosphonate was shown to reduce MDSC expansion in tumor and peripheral blood by inhibiting MMP-9 (8).

Regulatory Myeloid Cells as a Critical Component of Infection and Inflammation

It was reported that MDSC could down-regulate CD62L in naïve T cells, which in turn dampened the migration of naïve T cells to the lymph nodes. MDSC could suppress T-cell activation by sequestering cysteine. MDSC express the Xc transporter, which takes up cysteine, but lack the transporter required to export cysteine. Thus, they deprive T cells of cysteine, an essential amino acid required for their activation (9).

Tumor stroma has been shown to support tumor growth and development. Dr. Hans Schrieber (University of Chicago, Chicago, IL) suggested that targeting both the tumor stroma and the tumor itself might increase the efficacy of cancer therapy. In fact, if both stromal compartments (hematopoetic and mesenchymal) are presented effectively to T cells, tumor growth was greatly limited. It was proposed that if the tumor surrounding is destroyed completely, tumor growth and metastasis could be prevented.

The role of S100A8 and S100A9 proteins in inflammation has been appreciated for several years now. These proteins are involved in regulation of function of myeloid cells, specifically their migration to the site of injury. Recent data indicated that they could be involved in regulation of tumor progression (10).

Summary

The meeting concluded with a lively round table discussion in which different points of view on the regulatory component of MDSC in cancer and other diseases were critically evaluated. It was agreed that there is an urgent need for a more precise identification of the subsets of MDSC, especially in humans. The concept of expansion and activation of MDSC as two separate processes was discussed and found to be promising. It became increasingly clear that nonimmunological functions of MDSC are critically important components of tumor progression. It remained unclear, however, whether a substantial proportion of cells with a phenotype of MDSC contain immune-suppressive activity in the tumor site. It was agreed that functional definition of these cells required the presence of immune suppressive features. These issues need to be addressed in the near future. Little is known about the role of suppressive myeloid cells in physiological conditions. It needs to be clarified in order to understand the true immune regulatory role of these cells.

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No potential conflicts of interest were disclosed.

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