Cutting Edge: Mast Cells Critically Augment Myeloid-Derived Suppressor Cell Activity


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Myeloid-derived suppressor cells (MDSCs) are primarily recognized for their immunosuppressive properties in malignant disease. However, their interaction with other innate immune cells and their regulation of immune responses, such as in parasitic infection, necessitate further characterization. We used our previously published mouse model of MDSC accumulation to examine the immunoregulatory role of MDSCs in B16 melanoma metastasis and *Nippostrongylus brasiliensis* infection. In this study, we demonstrate that the activity of MDSCs is dependent on the immune stimuli and subset induced. Monocytic MDSCs predictably suppressed antitumor immune responses but granulocytic MDSCs surprisingly enhanced the clearance of *N. brasiliensis* infection. Intriguingly, both results were dependent on MDSC interaction with mast cells (MCs), as demonstrated by adoptive-transfer studies in MC-deficient (Kit<sup>Wsh</sup>/Wsh) mice. These findings were further supported by ex vivo cocultures of MCs and MDSCs, indicating a synergistic increase in cytokine production. Thus, MCs can enhance both immunosuppressive and immunosupportive functions of MDSCs. The Journal of Immunology, 2012, 189: 511–515.

Acute inflammatory reactions stimulate the development and recruitment of myeloid lineage cells, including neutrophils and macrophages. Unresolved inflammation increases myelopoiesis, shifting the distribution of myeloid subpopulations. This results in the premature bone marrow release of a heterogeneous population of mononuclear (CD11b<sup>+</sup>Ly6C<sup>high</sup>) and polymorphonuclear (CD11b<sup>+</sup>Ly6-G<sup>high</sup>) cells, known as myeloid-derived suppressor cells (MDSCs). These cells are widely studied in the context of neoplasia. MDSCs exert their proenoplasic effects through the release of small soluble oxidizers, the depletion of essential amino acids from the local extracellular environment, and the impairment of T cell/Ag recognition, all ultimately leading to T cell suppression (1). This MDSC-mediated immune suppression and impairment of cancer immunotherapy has been extensively investigated (2). However, by limiting immune activation, MDSCs can serve a beneficial role in transplantation, autoimmunity, and sepsis (3). This dual role of MDSCs depends upon NO production, the cytokine milieu, and the interaction among MDSCs, T cells, and NK cells (3–6).

Another interaction that may be physiologically significant is MDSC/mast cell (MC) collaboration. Although MCs have been well documented to mediate allergic inflammation, their demonstrated involvement in neoplastic disease is more recent. MCs were shown to accumulate in the tumor microenvironment and correlate with poor prognosis (7). This is largely due to the secretion of various proinflammatory cytokines produced by the MCs (8). Additionally, MCs contribute to MDSC recruitment to the tumor site via the production of 5-lipoxygenase and IL-17 (9, 10). Given that MC-deficient mice have weakened tumor growth and that MCs recruit MDSCs to the tumor microenvironment, it is necessary to further examine the contribution of this interaction to tumor metastasis. Furthermore, although several studies indicate the expansion, recruitment, and activity of MDSCs in parasitic infections, they do not clarify the extent to which MDSCs regulate this immune response (11).

Therefore, we analyzed the effect of MDSCs in anti-helminth immunity and B16 metastasis, as well as the interaction with MCs during the immune response. We used our previously published mouse model of MDSC accumulation in a tumor-free environment (12). In these mice, the overexpression of a disintegrin and metalloproteinase 10 (ADAM10) promotes the expansion of MDSCs that are analogous to tumor-induced MDSCs. Adoptive-transfer (AT)
studies using either C57BL/6 (wild-type [WT]) or MC-deficient KitWsh/Wsh mice were performed in parallel. Using two model systems, we demonstrate that the activity of MDSCs is variable, because granulocytic MDSCs enhanced antiparasite immunity, whereas monocytic MDSCs promoted B16 metastasis. Intriguingly, both activities depend upon the presence of MCs. This intercellular communication leads to augmentation of Th2-polarizing cytokines that are beneficial in the case of parasitic infection but maladaptive in neoplasia. Thus, our findings indicate that MDSCs have an immunomodulatory role that is largely dependent on the type of stimuli, the MDSC population involved, and the presence of MCs.

Materials and Methods

**Mice**

Mice were kept at Virginia Commonwealth University (VCU) in accordance with the humane treatment of laboratory animals set forth by the National Institutes of Health and the American Association for the Accreditation of Laboratory Animal Care. C57BL/6 ADA10 Tgs (A10Tgs) were generated from The Jackson Laboratory. All mouse protocols were approved by the VCU Institutional Animal Care and Use Committee.

**Cell lines**

The LLC cell line was obtained from the American Type Culture Collection. B16 melanoma and GM-CSF–B16 cells were provided by H.D.B. and maintained as previously described (12). Mouse bone marrow-derived MCs (BMMCs) were generated from mice through isolation and subsequent culture of bone marrow cells in complete RPMI 1640. The LLC cell line was used to generate monocytes from BM-derived MCs. LLCs were grown in high-glucose DMEM with 10% FCS and 10 ng/ml IL-3 over 4 wk. They were resuspended at 0.5 × 10^6 cells/ml, loaded with 0.5 μCi [3H]thymidine, and thymidine incorporation was measured.

**Isolation of MDSCs and AT**

Spleens were harvested from A10Tg or LLC-bearing WT mice, depending upon the experimental protocol. They were then dispersed into single-cell suspensions and filtered through 70-μm nylon mesh strainers (Invitrogen). Erythrocytes were lysed using an ammonium chloride potassium lysing buffer (Quality Biological). Gr-1+, Ly6G+, or Ly6C+ cells were purified from A10Tg spleens using the EasySep PE-Selection Kit (Stem Cell Technologies), according to the manufacturer’s protocol. For AT studies, 5–10 × 10^6 naive A10Tg MDSCs were injected into the tail vein of each experimental group. For T cell depletion, mice were injected i.p. with 200 μg anti-CD4 (GK1.5) and anti-CD8 (2.43) Abs on days −3, −2, −1, 0, 5, and 10.

**Arginase and NO detection**

MDSC cytoplasmic extracts were prepared from isolated A10Tg- and LLC-bearing hosts and tested for urea production (DARG-200; BioAssay Systems). NO was measured using Greiss Reagent (G7921; Molecular Probes). Cytospin preparations of splenic MDSCs were stained for L-selectin (CD-62L) and arginase 1 and inducible NO synthase (Supplemental Fig. 1D, 1E). The LLC cell line was used to generate monocytes from BM-derived MCs. LLCs were grown in high-glucose DMEM with 10% FCS and 10 ng/ml IL-3 over 4 wk. They were resuspended at 0.5 × 10^6 cells/ml, loaded with 0.5 μCi [3H]thymidine, and thymidine incorporation was measured.

**T cell suppression assays**

For polyclonal T cell activation, CD90.2+ T cells were sorted from spleen and activated with immobilized anti-CD3 (10 μg/ml) and soluble anti-CD28 (1 μg/ml) in 96-well plates. Sorted MDSCs (Ly6G+, Gr-1+, and Ly6C+) from A10Tg or LLC tumor-bearing mice were then added at increasing T cell/MDSC ratios. For Ag-specific T cell suppression assays, soluble gp100 (1 μg/ml) was added to defined ratios of pmel-1 transgenic splenocytes and A10Tg MDSCs. After 48 h of culture, 1 μCi [3H]thymidine was added to each well for an additional 18 h, and thymidine incorporation was measured.

**BMMC coculture with A10Tg MDSCs**

BMMCs were derived from femurs of WT naive mice cultured in complete RPMI 1640 containing 20% WEHI-3–conditioned medium over a 4-wk period. They were resuspended at 0.5 × 10^6 cells/ml, loaded with 0.5 μCi [3H]thymidine, and cultured with MDSCs at a 1:1 ratio in 10 ng/ml IL-3 and 10 ng/ml GM-CSF overnight. MCs were activated by DNP-human serum albumin (20–100 ng/ml), and supernatants were collected 6 or 18 h after cross-linking for ELISA.

**Adoptive immunotherapy of B16 melanoma**

T cells were prepared for adoptive immunotherapy (AIT), as previously described (13). Recipient mice were injected i.v. with 0.25 × 10^6 B16 cells. One day prior to AIT, mice were treated i.p. with 2 mg cyclophosphamide (CYP) and 1.2 mg gemcitabine (GEM) and were treated every 5 d with GEM only. After 14 d, lung nodules were counted, as previously described (13, 14); if too many to count, they were assigned a count of 250.

**N. brasiliensis infection, GEM treatment, and AT**

*N. brasiliensis* larvae were generously provided by Joe Urban (Agriculture Research Stabil, Beltsville, MD) and were maintained as previously described (15). Each experimental group of mice was infected with ∼650 stage 3 larvae (1.3) *N. brasiliensis*. Fecal egg burdens were enumerated on days 5–13, and serum was collected by tail vein nick. For mice injected with GEM, 1.2 mg was injected i.p. starting on day 0 and repeated every 5 d throughout the experiment. For AT studies, WT mice were injected with 5–10 × 10^6 naive A10Tg MDSCs via tail vein injection starting at day 0 of infection and repeated every 3 d thereafter. Additional groups of animals were sacrificed on day 7 postinfection, and the proximal halves of the small intestines were harvested. Adult worms were purified and enumerated.

**Statistical analysis**

The p values were calculated using unpaired two-tailed Student t tests or unpaired Mann–Whitney analysis. Error bars represent the SEM between samples.

**Results and Discussion**

MDSCs from A10Tg mice are phenotypically and functionally analogous to tumor-induced MDSCs

Although MDSC accumulation is a byproduct of ADAM10 overexpression in early hematopoietic progenitors, ADAM10 expression is not altered in these cells (12). To ascertain whether A10Tg-derived MDSCs are functional, ex vivo T cell suppression assays were performed. A10Tg MDSCs inhibited T cell proliferation of both A10Tg and WT purified T cells under polyclonal (Supplemental Fig. 1A, 1B) and Ag-specific (Supplemental Fig. 1C) conditions in a manner parallel to tumor-derived MDSCs (Supplemental Fig. 1D).

Tumor-derived MDSCs can be divided into granulocytic (CD11b+Ly6Ghigh) and more immunosuppressive monocytic (CD11b+Ly6Chigh) subsets (16). Accordingly, monocytic MDSCs possessed more suppressive potential. Furthermore, similar to T cells from tumor-bearing hosts, A10Tg T cells exhibited reduced levels of L-selectin (CD-62L), a receptor required for homing of naive T cells to the lymph nodes (Supplemental Fig. 1E, 1F) (17). MDSCs impair the T cell response through the activity of arginase 1 and inducible NO synthase. Accordingly, A10Tg MDSCs had levels of these enzymes that were comparable to tumor-derived MDSCs (Supplemental Fig. 1G, 1H) (2). These ex vivo observations indicate that MDSCs present in tumor-free A10Tg animals behave similarly to tumor-derived MDSCs.

**Monocytic MDSCs promote B16 metastasis**

We used the B16 melanoma model to examine in vivo activities of MDSCs from A10Tg mice. B16 was injected i.v.; although not a true metastasis, this allows comparison of tumor colonization into the lung with and without MDSCs and is commonly used to assess AIT (13). When challenged with B16, A10Tg animals were more susceptible to metastasis than were WT controls (Fig. 1A). The A10Tg animals had tumor nodules that were too numerous to count and were consequently assigned a count of 250. Parallel to the ex vivo...
assay, the suppressive activity of MDSCs was attributed to the monocytic population. When WT mice were challenged with B16 and AT of either monocytic or granulocytic MDSCs, melanoma metastasized more aggressively in mice given the monocytic subset, and granulocytic MDSCs had no effect (Supplemental Fig. 2A). AIT exerted a minimal effect on A10Tg mice, suggesting that the presence of MDSCs diminished the activity of adoptively transferred T cells. Although AIT treatment did not completely abolish metastasis in WT lungs, it induced a significant decrease in metastatic lesions. Given that lymphodepleting chemotherapy is known to enhance AIT (18), the protocol was modified to incorporate CYP and GEM. CYP lymphodepletes recipient mice, permitting homeostatic proliferation of transferred T cells; GEM preferentially decreases MDSC levels in tumor-bearing mice (19, 20). CYP and GEM alone and in combination in WT mice did not significantly alter metastasis, indicating that they have minimal effects on tumor cells (Supplemental Fig. 2C). However, GEM selectively diminished the MDSC population in A10Tg mice without affecting lymphocyte levels (Supplemental Fig. 2B). A10Tg mice treated with GEM alone exhibited an antitumor response comparable to WT counterparts, demonstrating that T cell function is restored upon MDSC depletion (Fig. 1B, 1C). Additionally, the combination of GEM, CYP, and AIT (tritherapy) resulted in complete regression of metastasis in both WT and A10Tg mice (Fig. 1B, 1C). Fig. 1D illustrates the significant abrogation of metastasis arising from tritherapy. Ideal AIT would use tumor-specific T cells and chemotherapy. Thus, the AIT protocol was adjusted to incorporate pmel-1 mice that are TCR transgenic for gp100 melanoma peptide (13). Similar results were observed, in that successful AIT required MDSC depletion (Supplemental Fig. 2C). Although not shown, A10Tg mice were also more susceptible to Lewis lung carcinoma compared with WT controls. Thus, MDSCs in A10Tg mice are functionally analogous to tumor-derived MDSCs, promote tumor metastasis, and compromise the efficacy of tumor immunotherapy.

Granulocytic MDSCs enhance the immune response against *N. brasiliensis*

Next, we examined the effect of MDSCs on the immune response in antiparasite immunity. Although MDSC accumulation following *N. brasiliensis* infection has been reported (11), the direct immunomodulatory role of MDSCs in anti-*N. brasiliensis* responses has not been examined. In *N. brasiliensis* infection, a WT response is characterized by production of Th2-associated cytokines that ultimately mediate adult worm clearance 10–12 d postinoculation (21). Following *N. brasiliensis* challenge, A10Tg animals had significantly reduced adult worm burden in A10Tgs (Fig. 2B). This indicated that MDSCs may facilitate parasite clearance. To determine whether the increased worm clearance correlated with an increase in Th2 cytokines, we examined a panel of Th2 cytokines. Analysis of peak infection (day 7) serum levels of IL-4, IL-5, and IL-13 demonstrated significantly enhanced levels in *N. brasiliensis*-infected A10Tg animals compared with infected littermates (Fig. 2C). Additionally, IL-17 and IL-33, which were reported to recruit MDSCs, were also elevated in serum of A10Tg mice (9, 22). These serum cytokines decreased as infection began to decline (data not shown).

To determine whether enhanced *N. brasiliensis* clearance is dependent on MDSC activity, MDSCs were depleted from A10Tg mice. GEM treatment resulted in elevated egg counts
in A10Tg mice similar to WT levels (Fig. 2A). Additionally, *N. brasiliensis* infection resulted in a modest 4–5-fold increase in MDSCs in WT spleens from days 0 to 14. This level of WT MDSCs is sufficient to promote *N. brasiliensis* clearance and prevent a chronic infection. However, GEM-mediated MDSC depletion in WT mice significantly exacerbated the peak level of *N. brasiliensis* infection (data not shown). Taken together, the data indicate that the enhanced immune response in A10Tgs is a consequence of elevated MDSC levels. Next, to eliminate off-target effects of GEM, A10Tg MDSCs were purified and adoptively transferred to WT mice over the course of *N. brasiliensis* infection. Like the MDSCs that were observed accumulating following *N. brasiliensis* infection, the transferred MDSCs also contained both Ly-6G+ and Ly6C+ populations. AT of MDSCs into WT mice significantly augmented both Ly-6G+ and Ly6C+ populations. AT of MDSCs into WT mice significantly exacerbated the peak level of *N. brasiliensis* infection (data not shown). Taken together, the data indicate that the enhanced immune response in A10Tgs is a consequence of elevated MDSC levels. Next, to eliminate off-target effects of GEM, A10Tg MDSCs were purified and adoptively transferred to WT mice over the course of *N. brasiliensis* infection. Like the MDSCs that were observed accumulating following *N. brasiliensis* infection, the transferred MDSCs also contained both Ly-6G+ and Ly6C+ populations. AT of MDSCs resulted in significantly reduced egg counts, comparable to A10Tgs (Fig. 2A). To determine which MDSC subset was responsible for this observation, MDSCs were purified based upon Ly6G and Ly6C expression and adoptively transferred into WT *N. brasiliensis*-infected mice. The Ly6Ghigh population had the same effect as did total Gr-1+–purified MDSCs, whereas the adaptively transferred Ly6Chigh population had no effect. This indicates that the granulocytic population of MDSCs mediates the anti-*N. brasiliensis* immunity (Fig. 2E). The direct role of MDSCs was further supported in WT mice that were T cell depleted. These mice exhibited the same rapid rate of clearance as WT adoptively transferred with MDSCs (Fig. 2A). As expected, *N. brasiliensis* clearance was significantly slower in T cell-depleted WT mice without AT of MDSCs (Fig. 2D).

**MCs are required for MDSC-mediated *N. brasiliensis* clearance**

Mucosal MC hyperplasia is a hallmark of gastrointestinal helminth infection that enhances the immune response, leading to parasite clearance (23, 24). Mice deficient in MCs exhibit delayed clearance kinetics (25). Given that MCs mainly produce Th2-polarizing and proinflammatory cytokines and that MCs chemoattract MDSCs, we examined whether the interaction between the two cell types could be contributing to the enhanced *N. brasiliensis* expulsion. Surprisingly, AT of MDSCs into MC-deficient mice did not enhance *N. brasiliensis* expulsion, indicating that an MDSC/MC interaction is critical for MDSC-mediated anti-*N. brasiliensis* responses (Fig. 2F).

To assess this interaction ex vivo, MCs were cocultured with MDSCs. This resulted in a synergistic increase in IL-6, IL-13, TNF-α, and MIP-1α (Fig. 3).

**MCs also contribute to MDSC-mediated immune suppression in B16 melanoma**

Given that MDSCs require MCs to enhance anti-*N. brasiliensis* immunity, the B16 metastasis study was re-evaluated in MC-deficient mice. Several groups demonstrated that MCs recruit MDSCs, which accumulate in the tumor microenvironment and correlate with poor prognosis. In addition, MC-deficient mice have decreased rates of tumor growth that increases to WT levels subsequent to MC reconstitution (26). Therefore, we anticipated that B16 melanoma cells would metastasize more slowly in *KitWsh/Wsh* mice compared with WT controls, but it would be enhanced with MDSC AT. *KitWsh/Wsh* and WT mice were injected with B16, with and without AT of MDSCs. WT mice were more susceptible to B16 metastasis compared with *KitWsh/Wsh* mice. Upon MDSC AT, WT mice exhibited similar levels of tumor nodules as did B16 A10Tgs, indicating the direct contribution of MDSCs to the immune response. However, much to our surprise, AT of MDSCs into *KitWsh/Wsh* mice failed to increase B16 metastasis (Fig. 4). Thus, these results demonstrate that MDSCs promote B16 melanoma metastasis in an MC-dependent manner and indicate that MDSC/MC interactions significantly enhance the MDSC-mediated suppression of antitumor immunity.

The A10Tg mouse affords a unique tool to characterize the immune-modulatory potential of MDSCs in an environment free of established tumor. Because the A10Tgs suffer no confounding pathology, this was an ideal system in which to elucidate the immunomodulatory roles of MDSCs. We
used this system to investigate roles for MDSCs in both neoplasia and helminth infection. In B16 metastasis, monocytic MDSCs strongly suppressed the immune response, whereas granulocytic MDSCs failed to promote metastasis. However, in *N. brasiliensis* infection, the granulocytic MDSCs enhanced the antiparasitic immune response, and the monocytic MDSCs had no effect. AT studies demonstrated that this differential immune regulation by MDSCs occurs in an MC-dependent manner. The ex vivo coculture studies indicate that MDSC/MC interactions may modulate immune responses by increasing cytokine production. MDSCs traffic to MCs, affording a synergistic Th2-skewed immune response. Although undesirable in the context of neoplasia, the observed response is ideal in helminth infections. Overall, our data indicate that inhibition of MC function may serve as a novel target to inhibit MDSC activity.

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**Disclosures**

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**References**


