Myeloid-Derived Suppressor Cells in the Pediatric Population

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♦ Introduction

Although mortality from childhood cancer is decreasing, cure remains elusive with certain tumors. Even with novel therapies, including immunotherapy, tumors continue to evade treatment. Within the tumor microenvironment, suppressor cells curb the immune system, including myeloid-derived suppressor cells (MDSCs)—a heterogeneous population of immature myeloid cells which play a role in cancer progression and metastasis. Two subsets of these cells—granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs)—promote this immunosuppressive milieu by inhibiting anti-tumor T cell activity. We have investigated the functional properties of MDSCs induced in vitro from a pediatric medulloblastoma line and explored the mechanism of T cell suppression from these cells.

♦ Methods & Materials

We induced M-MDSCs by co-culturing donor monocytes with medulloblastoma cells in 6-well and transwell plates and identified them via flow cytometry. By activating the donor's T cells with CD3, CD28 beads, staining them with CFSE, and plating them with MDSCs, we could discern via flow cytometry if these MDSCs are T cell suppressive. We used intracellular DCFDA to stain the MDSCs for reactive oxygen species (ROS). The genetic profile of these MDSCs compared to donor monocytes was analyzed by the University of Louisville Genomics Core. We confirmed the differences via real-time PCR. We assessed the cytokine profile of the tumor cells and again confirmed results by ELISAs. To confirm our mechanistic pathway, we compared healthy monocyte lysate to MDSCs via western blot.

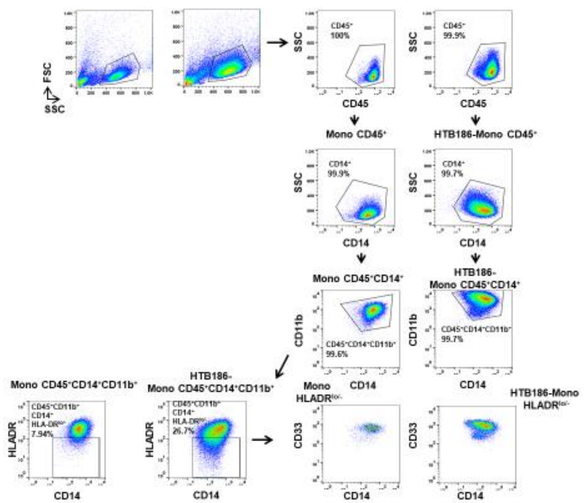

Figure 1. Pediatric Medulloblastoma-Educated CD14+ Monocytes Acquire an MDSC Phenotype Via a Soluble Factor. 26.7% M-MDSC population in transwell co-cultured cells compared to 7.94% in healthy donor monocytes signifying induction is via a soluble factor.

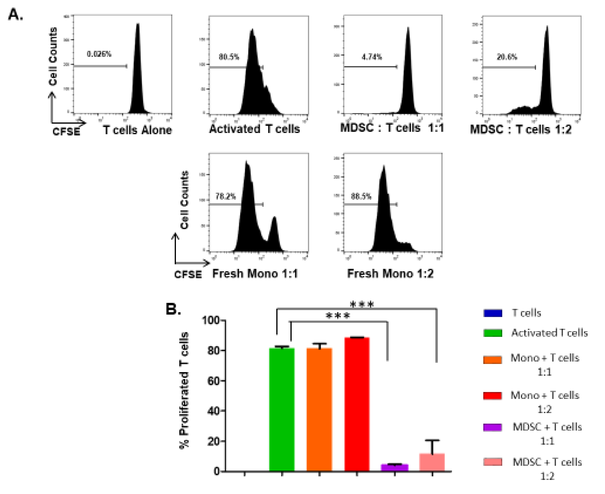

Figure 2. Transwell Medulloblastoma-Educated MDSCs are Potent Suppressor of T cells. Adding MDSCs to activated T cells inhibits proliferation whereas no inhibition is seen with the addition of fresh, autologous monocytes.

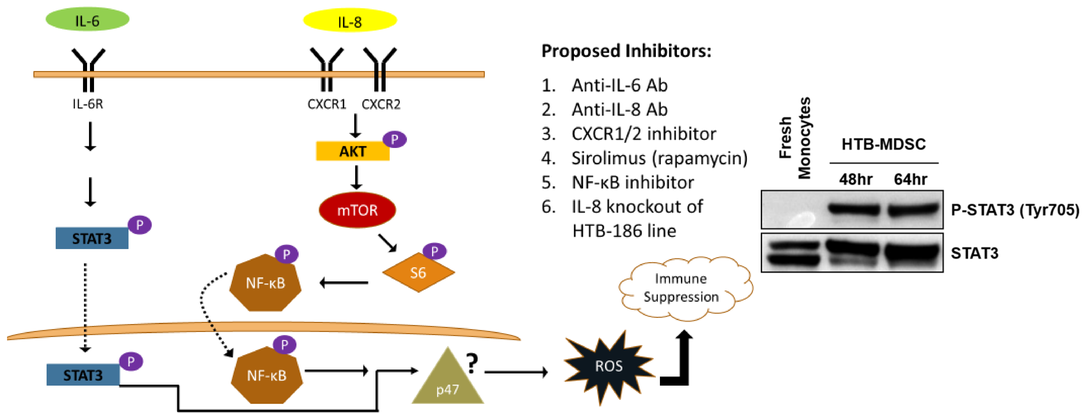

Figure 3. Medulloblastoma-Induced M-MDSCs Increase ROS via IL-6 and IL-8 Pathways. Our hypothesized mechanism of T cell suppression by medulloblastoma-induced M-MDSCs, confirmed via Western blot. We have proposed to use a series of inhibitors to further elucidate this pathway.

♦ Results

In the co-cultured monocytes, we found an increased population of CD14+, CD11b+, CD33+, HLA-DR low cells—a signature specific for M-MDSCs. Induction was present in the 6-well and transwell plates, signifying induction is via a soluble factor (Fig 1). These MDSCs proved T cell suppressive as T cell proliferation decreased with MDSCs (Fig 2). The MDSCs showed greater DCFDA expression, indicating increased ROS in the MDSCs. Adding PMA to these cells increased levels of ROS. Our microarray data showed almost a 500-fold increase in NOX4 gene expression in MDSCs. The cytokine array on the medulloblastoma cells showed large amounts of IL-6 and IL-8 release. We confirmed our mechanistic pathway of immunosuppression via Western blots with increased signal in activated STAT3, AKT, S6, and NF-ƙB in MDSC lysates (Fig 3).

♦ Conclusion

We show that medulloblastoma cells induce T cell suppressive M-MDSCs in vitro and believe this suppression is by ROS, beginning with the large quantity of IL-6 and IL-8 produced by these tumors. IL-6 is known to increase levels of STAT3, leading to increased levels of ROS. However, the role of IL-8 is less clear. We believe IL-8 activates AKT, activating ribosomal protein S6 via mTOR. This increases activation of NF-ƙB, also known to be active in MDSCs. It is yet unclear whether these systems are acting separately or together to increase ROS and cause immunosuppression. We have proposed using multiple inhibitors and an IL-8 knockout tumor line to further elucidate this pathway (Fig 3). We hope that these inhibitors may offer novel treatment for medulloblastoma or that IL-6 or IL-8 may act as prognostic indicators for patients with this disease.