GLIOMA-DERIVED FACTORS INDUCE AN IMMUNE SUPPRESSIVE PHENOTYPE IN BONE MARROW-DERIVED CCR2+ MYELOID CELLS


Background Glioblastoma (GBM) is an aggressive primary brain tumor that is highly resistant to immune checkpoint inhibitors (ICIs). Bone marrow-derived myeloid cells comprise a large proportion of glioma-infiltrating leukocytes and are associated with an immune-suppressive phenotype. We previously characterized monocytic-myeloid derived suppressor cells (M-MDSCs) based on expression of the chemokine receptors CCR2 and CX3CR1. Targeting CCR2, in combination with anti-PD-1, reduced M-MDSCs in the tumor microenvironment and prolonged survival in ICI resistant murine gliomas, KR158B and 005GSC. We also established that M-MDSCs migrate to CCR2 ligands, CCL2 and CCL7, in a redundant manner. Dual targeting of CCL2 and CCL7 limited the presence of CCR2+/CX3CR1+ M-MDSCs in the glioma microenvironment. While knowledge of the mechanisms that recruit myeloid cells to the glioma microenvironment is better understood, it remains unclear what factors drive the myeloid-derived immune suppressive phenotype in GBM. The objective of this study is to evaluate the effect of glioma-derived factors on immature myeloid cells.

Methods C57BL/6 mice were implanted with KR158B glioma cells. Brain tumor tissue was subjected to Luminex cytokine analysis and compared to healthy brain. Bone marrow from Ccr2RFP/WT/Cx3cr1GFP/WT mice was cultured in the presence of KR158B glioma conditioned media followed by fluorescent microscopy and flow cytometry analysis to assess M-MDSCs. T cell suppression assay was conducted using Ly6G-/GR1+ cells (M-MDSCs) isolated from bone marrow cultures supplemented with KR158B conditioned media and freshly isolated T-cells to examine their immune-suppressive phenotype. In vivo iNOS was examined by flow cytometry and immunohistochemical analyses. NOS inhibitor, L-NMMA, was used to evaluate the role of nitric oxide in M-MDSC-mediated T cell suppression.

Results KR158B gliomas differentially upregulate a number of cytokines/growth factors, including CCL2, IL6, G-CSF, GM-CSF, as compared to healthy naïve brains. KR158B conditioned media increased the percentage of bone marrow-derived CCR2+/CX3CR1+ cells that express M-MDSC markers. Bone marrow-derived CCR2+/CX3CR1+ cells, expanded in KR158B condition media, suppress both CD4+ and CD8+ T cell proliferation. M-MDSCs in KR158B glioma microenvironment regulate iNOS compared to M-MDSCs present in the bone marrow. NOS inhibitor, L-NMMA, prevented M-MDSC suppression of CD8 T cell proliferation, but had no effect on suppression of CD4 T cells.

Conclusions Glioma-derived factors recruit and induce CCR2+/CX3CR1+ myeloid cells to a CD4/8+ T cell suppressive state that can be partially ameliorated by iNOS inhibition. While additional M-MDSC-mediated immune suppressive mechanisms need to be identified, targeting M-MDSCs holds promise as an effective approach to improve immune-directed interventions in GBM.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0987