A TL1 Team Approach to Integrating Mathematical and Biological Models to Target Myeloid-Derived Immune Cells in Glioblastoma

***Gregory P. Takacs[1], *Hannah Anderson[2], Christian Kreiger[1], Defang Luo[1], Libin Rong[2], Tracy Stepien[2], Jeffrey K. Harrison[1]**

[1] Department of Pharmacology & Therapeutics, University of Florida College of Medicine, Gainesville, FL

[2] Department of Mathematics, University of Florida College of Arts and Sciences, Gainesville, FL

*Contributed equally to this work

QR code directs to Flores-Toro et al. PNAS 117:1129, 2020

Abstract

Objective: The goal of this team approach is to integrate mathematical models of glioblastoma (GBM) infiltrating myeloid cells that contribute to the immunosuppressive phenotype in glioma with experimental data to predict therapeutic responses to combined chemokine receptor and immune checkpoint blockade.

Methods: Orthotopic murine KR158-luc gliomas were established in fluorescent reporter CCR2$^{WT/RFP}$, CX3CR1$^{WT/GFP}$ mice. Subsequently, an anti-CD31 injection was administered to label the vasculature. Fluorescent imaging and quantification of anti-CD3 stained sections were performed on a range of tumor sizes to acquire vasculature, tumor, T cell, and myeloid cell densities. In parallel, a system of ordinary differential equations (ODEs) was formulated based on biological assumptions to evaluate the change over time of tumor cells, T cells, and infiltrating myeloid cells. The model is then refined and validated by experimental results.

Results: Fluorescent imaging and quantification revealed a correlation between tumor size and abundance of (CX3CR1^{+}, CCR2^{-}) and (CX3CR1^{+}, CCR2^{+}) myeloid cell populations in the tumor microenvironment (TME). The density of these cell populations and vasculature remained constant as the tumors increased in size. Computer simulations of the mathematical model will predict tumor, myeloid, and T cell dynamics. These simulations will be particularly useful to uncover information regarding myeloid cell dynamics. Parameter sensitivity analysis of the model will inform us of the biological processes driving these tumor-immune cell dynamics.

Discussion: GBM is a challenge as current interventions are ineffective. This study improves the understanding of glioma infiltrating myeloid cells and their impact on tumor progression. The data will serve as a basis for quantitatively predicting therapeutic responses of a novel combination treatment.

Background

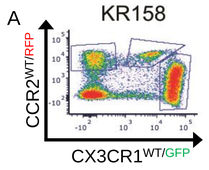
Chief amongst the immune-suppressive cells that gain access to human gliomas are a subset of myeloid derived cells, termed myeloid derived suppressor cell (MDSCs). MDSCs contribute significantly to the PD-L1/PD-1 resistant phenotype observed in gliomas [1-7]. Previously, we have determined that glioma-associated myeloid cells can be distinguished by their chemokine receptor (CCR2 and CX3CR1) expression patterns [8]. Our results also established that pharmacologic targeting of CCR2-expressing myeloid derived suppressor cells (MDSCs) unmasked an effect of PD-1 blockade to slow progression of two immune checkpoint inhibitor-resistant murine gliomas. Flow cytometry analysis demonstrated that CCR2 antagonism limited the presence of MDSCs cells within the tumor and promoted their bone marrow sequestration [8].The current study evaluated myeloid-derived immune cell dynamics within the glioma microenvironment with the goal of generating mathematical models that account for tumor-immune dynamics.

References: 1. Raychaudhuri B, et al. (2011) Neuro Oncol 13:591-599, 2. Raychaudhuri B, et al. (2015) J Neurooncol 122:293-301, 3. Kohanbash G, et al. (2013) Cancer Res. 73:6413-6423, 4. Prosniak M, et al. (2013) Clin Cancer Res.19:3776-3786, 5. Chae M, et al. (2015) Neuro Oncol 17:978-991, 6. Gielen PR, et al. (2015) J Neuropathol Exp Neurol 74:390-400, 7. Chang AL, et al. (2016) Cancer Res; 76; 5671–5682, 8. Flores-Toro, *et al*. (2020) PNAS 117:1129.

Methods

Orthotopic murine KR158-luc gliomas were established in fluorescent reporter CCR2$^{WT/RFP}$,CX3CR1$^{WT/GFP}$ mice. Subsequently, an anti-CD31 injection was administered to label the vasculature. Immunohistochemical (IHC) fluorescent imaging and quantification of anti-CD3 stained sections were performed on a range of tumor sizes to acquire vasculature, tumor, T cell, and myeloid cell densities. In parallel, a system of ODEs was formulated based on biological assumptions and in comparison with experimental results. Numerical simulations including bifurcation and sensitivity analysis, and their biological implications will be further discussed.

Tumor microenvironment in KR158 implanted CCR2$^{WT/RFP}$;CX3CR1$^{WT/GFP}$ mice



B

CX3CR1^{+}: Microglia
CD45^{hi}/CD11b^{mid}/Ly6C^{-}/Ly6G^{-}
MHCII^{+}/F4/80^{+}/CD11c^{-}

CCR2^{+}/CX3CR1^{+}: MDSCs
CD45^{hi}/CD11b^{hi}/Ly6C^{hi}/Ly6G^{-}
MHCII$^{-/+}$/F4/80^{+}/CD11c^{+}

CCR2^{+}: Unclear
CD45^{hi}/CD11b^{inter} /Ly6C^{-}/Ly6G^{-}
MHCII^{-}/F4/80^{-}/CD11c^{+}

(Flores-Toro *et al.* PNAS 117:1129, 2020)

***Figure 1: (A)** Flow cytometry plot of tumor isolates from KR158 implanted CCR2$^{WT/RFP}$;CX3CR1$^{WT/GFP}$ mice. **(B)** Flow cytometric cell marker analysis of the three distinct CCR2 and CX3CR1 expressing cell populations.*

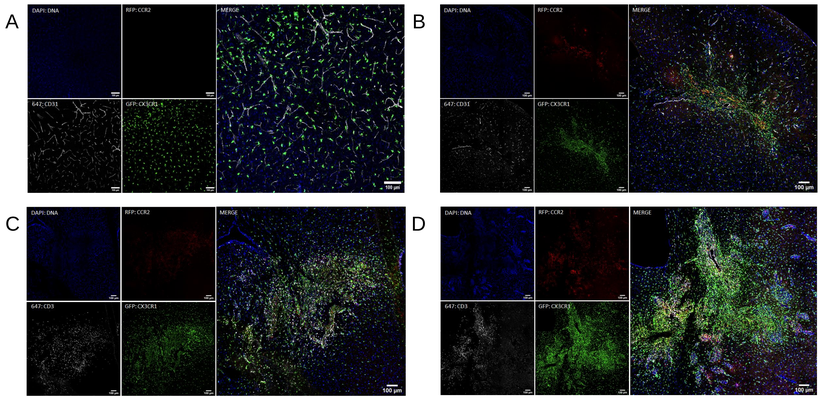


***Figure 2:** Representative fluorescent immunohistochemical (IHC) images from naive and tumor bearing KR158 implanted CCR2$^{WT/RFP}$;CX3CR1$^{WT/GFP}$ mice. **(A)** Normal brain parenchyma **(B-C)** Tumor infiltrating CCR2,CX3CR1 expressing cells in the context of anti-CD31 vascular labeling or anti-CD3 T-cell labeling. **(D)** Spatial localization of CCR2 and CX3CR1 expressing cells in TME.*

Summary

- Flow cytometry identifies three populations of CCR2 and CX3CR1 expressing cells in KR158 murine gliomas.
- Normal brain parenchyma of CCR2$^{WT/RFP}$ CX3CR1$^{WT/GFP}$ tumor bearing mice displayed equally distributed CX3CR1 expressing cells and anti-CD31 staining-no infiltrating CCR2 cells.
- CCR2 and CX3CR1 expressing myeloid sub populations were localized to distinct, although sometimes overlapping, regions of the tumor microenvironment (TME).
- CCR2^{+}/CX3CR1^{+} , CX3CR1^{+} , CD3^{+} cells accumulate in the TME with increase tumor burden.
- The ODE model will be fitted to the data and used to run computer simulations in order to develop predictions of progression.

This work supported in part by the NIH/NCATS Clinical and Translational Science Awards to the University of Florida UL1TR001427 and TL1TR001428

CCR2^{+}/CX3CR1^{+} , CX3CR1^{+} , CD3^{+} cells accumulate in the TME

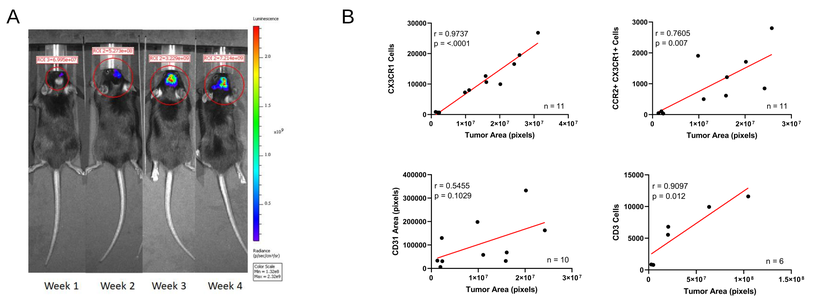


***Figure 3: (A)** Luciferase expressing KR158 glioma cells were orthotopically implanted and stratified using bioluminescent signal (surrogate for tumor burden).**(B)** Pearson's correlation analysis was conducted on cell abundance and tumor area from fluorescent IHC images.*

Mathematical model of tumor-immune dyamics

A

Rate of change of Tumor cells =

$$\frac{dC}{dt} = \underbrace{\lambda_C C\left(1-\frac{C}{C_K}\right)}_{\text{logistic growth}} \underbrace{-\eta T C}_{\text{killed by T cells}}$$

Rate of change of T cells =

$$\frac{dT}{dt} = \Big(\underbrace{a_{I12}}_{\text{activation by IL-12}} + \underbrace{s_{I2}T}_{\text{stimulation by IL-2}} \Big) \underbrace{*F_1(Q)}_{\text{inhibition by PD-1-PD-L}} \underbrace{-rTM}_{\text{inhibition by MDSCs}} + \underbrace{j\frac{C^2}{k+C^2}T}_{\text{tumor recruitment of T cells}} \underbrace{-d_T T}_{\text{death}}$$

Rate of change of MDSCs =

$$\frac{dM}{dt} = \underbrace{s_{M2} * F_2(C)}_{\text{stimulation by CCL2}} + \underbrace{s_{M7} * F_3(C)}_{\text{stimulation by CCL7}} - \underbrace{r_{I12}M}_{\text{inhibition by IL-12}} + \underbrace{\alpha\frac{C}{q+C}}_{\text{expansion of MDSCs}} \underbrace{-d_M M}_{\text{death}}$$

B

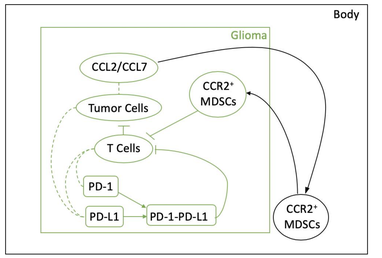


***Figure 4: (A)** The ODE model created to describe the change over time of the tumor microenvironment (TME).*
***(B)** A diagram of the tumor-immune dynamics outlined in the mathematical model.*

References: 9. Nikolopoulou et al. (2018) Lett. Biomath. 5(17):1-23. 10. Shariatpanahi et al. (2018) J Theor Biol. 442:1-10.

Conclusion

- CCR2^{+}/CX3CR1^{+} cells accumulate in the tumor microenvironment as tumor burden increases, supporting the rational that a CCR2 antagonist may be used at any stage of tumor burden.
- Future directions entail modeling combination PD-1 immune checkpoint blockade and CCR2 antagonist paradigm in murine models and the mathematical model with the goal of translating to the human condition.