CD8+CD28- T cells contribute to the immunosuppressive tumor microenvironment of malignant glioma

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Disclosure

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Introduction

• Malignant gliomas are the most frequent primary brain tumors with poor overall prognosis and no significant improvements in treatment for several decades. Immunotherapy for gliomas has been under much investigation.

• Antigen-specific CD8+CD28- T cells are considered terminally differentiated, arising from CD8+CD28+ T cells after multiple cell divisions, with both suppressor and cytotoxic subsets. In vitro studies reported CD28 downregulation in the presence of IL-2, IL-7, and IL-15, while IL-10 and TGF-β stimulated induction and expansion of CD8+ regulatory T cells.

• Conflicting studies of several autoimmune diseases and various cancers have shown CD8+CD28- T cells associated with both cytotoxic and immunosuppressive activities. However, the role and development of CD8+CD28- T cells in malignant glioma has not yet been examined.
Methods

• Male mice were anesthetized and intracranially injected with GL261, GL261-CMV, or GL261-OVA malignant glioma cells and euthanized, in addition to normal control group, at 1 and 3 weeks post-intracranial injection (WPO) or at humane endpoint determined by signs of distress.

• We used qPCR to measure IL-2, IL-7, IL-15, IL-10, and TGFβ levels in brain and flow cytometry to measure CD8+CD28- T cells in brain and to assess their functional intracellular cytokines and cytotoxic capacities.

• Human glioblastoma (GBM) and blood samples were collected after patient consent at Methodist Hospital - Indiana University Health.

• Using UCSC Xena Browser TCGA database, graphs were created of IL-2, IL-7, IL-15, IL-10, and TGFβ levels in brain samples from healthy controls and GBM patients. We used flow cytometry human GBM and blood samples to measure the CD8+CD28- T cells in brain and to assess their functional intracellular cytokines and cytotoxic capacities.
Results

CD8+CD28+ and CD8+CD28- Expression in Human Glioma Patients

(A) Flow cytometry of human GBM CD8+CD28- T cells.

(B) UCSC Xena graphs of cytokines expressed within human malignant glioma.

**FIGURE 1.** (A) Flow cytometry of human GBM CD8+CD28- T cells. (B) UCSC Xena graphs of cytokines expressed within human malignant glioma.
Results

FIGURE 2. (A) Flow cytometry of mouse malignant glioma CD8+CD28- T cells. (B) qPCR of extracellular IL-2, IL-7, IL-15, IL-10, and TGF-β in mouse malignant glioma.
FIGURE 3. Flow cytometry of intracellular IL-10, TGF-β, IFN-γ, and TNF-α from mouse malignant glioma CD8+CD28- T cells.
Results

FIGURE 4. Flow cytometry of surface CD107, PD1, TIM-3, & LAG-3 from mouse malignant glioma CD8+CD28- T cells.
Discussion

• A fraction of human malignant glioma infiltrating CD8+ T cells are CD8+CD28- T, which appeared higher in brain compared to blood of human GBM patients and in our mouse model of malignant glioma at 1WPO.

• The USCS Xena Browser TCGA database suggested increased levels of IL-2, IL-7, IL-15, IL-10, and TGFβ levels in brain samples from GBM patients. IL-2 and IL-7 appeared less likely predominant factors in downregulation of CD28, while IL-15 showed an increasing trend in GL261 mice compared to control along with increasing trends of IL-10 and TGFβ in ME. Further work remains, such as protein analysis.

• CD8+CD28- T cells showed an increasing trend of intracellular (IC) TGFβ in GL261 mice, while IC IFNγ and TNFα disappeared by 3WPO. CD107a, TIM-3, and LAG-3 did not show significant increases in brain CD8+CD28- T cells. Significant increases of PD1 occurred in CMV- and OVA-GL261 CD8+CD28- T cells with an increasing trend in GL261.
Summary Points

• CD8+CD28- T cells may have an immunosuppressive effect in the TME.

• A fraction of malignant glioma infiltrating CD8+ T cells are CD8+CD28- T, and appeared higher in brain compared to blood.

• Tumor derived cytokines may regulate CD28 expression on CD8+ T cells and the phenotype of CD8+CD28- T cells.

• We concluded that glioma ME actively manipulates tumor infiltrating cytotoxic CD8+ T cells.

• Blocking conversion of CD8+CD28+ T cells into CD8+CD28- T cells in the tumor ME may enhance the efficacy of anti-glioma immunotherapy.