The Effect of Temozolomide on T cell function in GBM and Implications for Immunotherapeutic Strategies

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DISCLOSURE STATEMENT

The authors have nothing to disclose.
INTRODUCTION

BACKGROUND & SIGNIFICANCE
Patients with cancer are often severely immunosuppressed at the time of diagnosis, with glioblastoma (GBM) patients being no exception to this finding. Specific evidence of immunosuppression includes upregulation of exhaustion markers Tim3, PD-1, and CD39 on peripheral blood T cells. This immune suppression which is a product of the malignancy itself is further exacerbated by the standard treatment for GBM, temozolomide (TMZ), which has been shown to cause lymphopenia and decreased T cell function. The lymphopenia induced by TMZ potentiates the response to cellular immunotherapy and promotes expansion of antigen-specific T cells. However, TMZ appears to have a detrimental effect on immune checkpoint inhibition by undermining the immune response generated by a PD-1 blockade. Therefore, TMZ appears to have a beneficial effect on the exogenous immune response (adoptive T cell transfer) while hindering the endogenous immune response (immune checkpoint inhibition) in GBM.
METHODS

Our objective is to understand the differing roles of TMZ in the exogenous vs endogenous immune response in order to understand its effect on differing types of immunotherapy. Our hypothesis is that TMZ-induced T cell exhaustion leads to worse outcomes for GBM patients, dosing can influence the degree of induced exhaustion, and immunotherapeutic strategies (dendritic cell vaccine) can reverse T cell exhaustion.

SPECIFIC AIMS

1. To determine the extent of lymphopenia induced by different dosing strategies of Temozolomide. Using a syngeneic murine glioma model, we will determine the effect of Temozolomide treatment on immune cell production.

2. To determine the extent of T cell exhaustion in GBM and the specific mechanism by which Temozolomide further exacerbates T cell exhaustion. Using a syngeneic murine glioma model, we will evaluate cytokine production as a measure of T cell function and investigate the upregulation of established exhaustion markers.

3. To investigate the extent to which immunotherapy can reverse Temozolomide-induced T cell exhaustion in GBM. Dendritic cell vaccine will be administered concurrently with differing combinations of temozolomide and immune check point inhibitors in an attempt to curtail exhaustion.
RESULTS

C57BL/6 mice were treated with two different doses of TMZ - Standard or Metronomic. Standard dose (SD) consisted of 50mg/kg TMZ for five days. Metronomic dose (MD) consisted of 25 mg/kg TMZ for ten days. Blood was drawn from animals 1-, 2-, and 6- weeks post-administration. The numbers of CD4+ and CD8+ peripheral blood T cells were measured over time. From these measurements, it was confirmed that TMZ induces lymphopenia. In both SD and MD groups, the numbers CD4+ and CD8+ T cells declined from baseline; however,
the SD group experienced the sharpest and most dramatic fall in T cells. Therefore, this experiment determined that not only does TMZ induce lymphopenia, but does so in a dose-dependent manner.

Tim3 and Lag3 are well-known markers of dysfunctional or exhausted T cells in the context of malignancy. Utilizing ELISA, we determined that TMZ increases Tim3 and Lag3 expression in a dose-dependent fashion, with standard dose TMZ increasing expression of these markers in both CD4+ and CD8+ T cells over a two week period. MD was unable to produce significant upregulation of Tim3/Lag3 in either types of T cells.
IFN-gamma serves several critical functions in modulating the immune response, namely in assisting in the proliferation of CD4+ and CD8+ T cells. Therefore, IFN-gamma secretion is a reliable marker of T cell health. Following administration of SD and MD, an ELISA kit was used to gauge IFN-gamma secretion by T cells. Both groups revealed reductions in IFN-gamma secretion, with SD inducing the greatest degree of reduction.
The use of dendritic cells (DCs) as a means of reversing malignancy-induced T cell exhaustion is not a novel concept. DCs help prime, activate, and direct T cells to target tumor cells. This experiment attempted to reverse SD TMZ-induced T cell exhaustion through administration of DCs co-cultured with tumor antigen and given either before or after TMZ administration. As shown above, DC vaccination did not significantly improve IFN-gamma secretion by T cells in animals given TMZ. Therefore, DCs did not help reverse TMZ-induced T cell exhaustion. Further investigation is required to determine alternative methods of reversing exhaustion.
DISCUSSION

CONCLUSIONS We began this investigation with the intent of uncovering peripheral blood markers of TMZ-induced immune suppression, understanding the mechanisms of TMZ-induced T cell exhaustion, and developing methods to overcome exhaustion. We discovered that markers of immune suppression include upregulated Tim3 and Lag3 on CD4+ and CD8+ T cells. We revealed that the effect of TMZ on T cell exhaustion is dose-dependent, with standard dosing regimens have a more detrimental effect on functional T cell counts compared with metronomic dosing. Unfortunately, we were unable to reverse TMZ-induced exhaustion using (DC vaccination). Further study is required to devise methods of overcoming T cell exhaustion.
SUMMARY OF KEY POINTS

- TMZ induces lymphopenia, increases expression of T cell exhaustion markers Tim3 and Lag3, and decreases IFN-gamma secretion in a dose-dependent manner.

- Dendritic cells co-cultured with tumor antigen were not able to reverse TMZ-induced T cell exhaustion.

- Further study is required to develop means of reversing T cell exhaustion secondary to treatment with TMZ.