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CD8+ T-Cell Infiltrate in Newly Diagnosed Glioblastoma is Associated with Long-Term Survival

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Abstract

A growing body of evidence supports the significant interplay between the immune system and glioma pathogenesis. Here we investigate whether the extent of local glioma-associated CD8+ T-cell infiltrate at initial presentation correlates with long-term survival in patients with glioblastoma multiforme (GBM). The study was conducted by the University of California San Francisco Brain Tumor Research Center as part of the San Francisco Bay Area Adult Glioma Study, which included over 519 patients with GBM. A central neuropathology review was performed and populations of infiltrating CD8+ T-cells were quantified histologically. Of 108 patients studied, 43 patients had poor survival (< 95 days) and 65 patients had extended long-term survival of > 403 days. Tumors from long-term survivors were more likely than short-term survivors to have intermediate or extensive T-cell infiltrates compared to focal or rare infiltrates, and this association appears to be most significant in Caucasian women ($p < 0.006$). Thus, CD8+ T-cell infiltrate is associated with prolonged survival. Our data provide the impetus for more sophisticated studies to further elucidate prospectively the specific T-cell subtypes associated with long-term survival.

Keywords

Clinical outcome; Glioma; Glioblastoma; Immune infiltrate; T-cell infiltrate

1 Introduction

A growing body of evidence supports the significant interplay between the immune system and glioma pathogenesis with clinical implications [1]. Long-term remission of malignant brain tumors secondary to post-operative infection has generated the hypothesis that a heightened immune status can confer some protection against intracranial tumors [2]. Several reports have also suggested that a significant allergy history lowers an individual's lifetime risk for developing an intracranial glioma [3,4]. In contrast, an impaired immune system may generate a state in which intracranial tumors may more easily develop. This is suggested by the noted correlation between human immunodeficiency virus-mediated

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immunosuppression and intracranial glial tumors [5-7]. Furthermore, iatrogenic immunosuppression associated with transplant recipients have also been implicated in the development of intracranial glioma [8,9].

Serologic analysis of antigens using recombinant cDNA expression cloning (SEREX) has identified several tumor-associated antigens that are able to generate a specific response in a variety of human cancers, including malignant glioma [10,11]. These finding suggest that a T-cell dependant immune response may improve the outcome of glioma patients through an antigen-mediated immune response (Fig. 1).

Cytotoxic T-cell (CD8+ T-cell) infiltrates found in glioma tissue may represent a local antigen-dependant immune activation against the glioma. Our hypothesis in this investigation was that the extent of CD8+ T-cell infiltrate at initial presentation and diagnosis correlates with long-term survival in patients with GBM. CD8+ T-cell infiltration in newly diagnosed glioma patients have not been well characterized in relation to long-term survival; here we report our specific analysis of 108 patients evaluated for CD8+ T-cell infiltrate and long-term survival.

2 Materials and Methods

2.1. Patient population

The study was conducted by the University of California San Francisco (UCSF) Brain Tumor Research Center as part of a population-based study, the San Francisco Bay Area Adult Glioma Study from 1991 to 1994 and 1997 to 1999, which included over 519 patients with GBM [4,12,13]. During these time frames, this study attempted to enroll every adult patient with glioma in the San Francisco Bay Area (as classified by the International Classification of Disease for Oncology [4,12,13], morphology codes 9380-9481). Utilizing the Northern California Cancer Center's rapid case ascertainment system, adult patients with glioma in the Bay Area were ascertained within 2 to 8 weeks after diagnosis. The Northern Californa Cancer Center is part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Treatments, vital status, and other factors were determined utilizing registry, medical records, interviews, and active follow-up data [13]. These protocols have been previously described [4,13,14]. From these survival data, those patients with a survival < 95 days (n = 43) and those > 403 days (n = 65) were all further evaluated for prolific CD8+ T-cell infiltration. All study protocols were reviewed by the Committee on Human Research at UCSF.

2.2. Immunohistochemistry and Histological Analysis

All pathology records and specimens were obtained and reviewed by a central neuropathology review [13]. Central neuropathology review was performed based on the World Health Organization (WHO) II Classification [13,15]. Quantification of proliferating CD8+ T-cell infiltration was performed histologically by systematically screening the entire tumor area from at least 3 sections obtained from different portions of the glioma. Ten microscopic fields were then chosen and examined with histological fluoroscopy. The average numbers of > 50 CD8+ T-cell infiltrates were noted as infiltration and these groups were classified into minimal, intermediate and extensive infiltration according to this distinction. CD8+ T-cell infiltrates were identified through immunohistochemistry using acetone-fixed cryostat sections stained with hematoxylin and eoin (H&E) followed by monoclonal antibodies against human CD8+ (AbCam; Cambridge, MA, USA). Cutoffs for survival quartiles included 95 days, 214 days, and 403 days. Mitigating factors known to affect survival were controlled for, including age, extent of resection, and adjuvant therapies. The neuropathology reviews of the tissue samples were performed in a blinded

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fashion in regards to long-term survival. Because the specific hypothesis of this study was to compare long-term survivors against short-term survivors, we specifically analyzed the CD8+ T-cell infiltrate in a bimodal manner by evaluating the short-term survivors and the long-term survivors based on our previously published quartile survival data.

2.3. Statistical analysis

The raw data were tabulated using Microsoft Excel (Microsoft; Seattle, WA, USA). All results were analyzed using the chi-squared (χ^2) test, Fisher's exact probability test or a *t*-test when appropriate for statistical evaluation of the data. A multivariate analysis of CD8+ T-cell infiltration adjusting for known prognostic and treatment factors was performed using the logistic regression model. For all statistical investigations, tests for significance were 2-sided, with a (2-tailed) *p* -value threshold of 0.05 considered statistically significant. Unless otherwise stated, all continuous values were presented as mean ± standard deviation (SD).

3. Results

3.1. Patient Demographic Results and Censoring

A total of 519 patients were enrolled in the San Francisco Bay Area Glioma SEER study with central neuropathology review. The median age at diagnosis in this group was 63 years of age. The median length of survival was 7.2 months with a 95% confidence limit of 6.6 to 7.9 months (Table 1). Not all ptient slides could be stained and evaluated, and were censored from analysis. Using the determined cutoffs of poor long-term survival at < 95 days ($n = 43$) and the extended long-term survival of > 403 days ($n = 65$), 108 samples were analyzed. Of the patients whose samples were analysed, the mean age was 65.2 years in the short-term survivor group and 54.3 years in the long-term survivor group (Table 1).

The characteristics of our patient population in this study reflected the regional demographics of the study population in the San Francisco Bay Area. Of the study samples, 44% were female, and 83% of the patients enrolled in this study were White or Caucasian in ethnicity. This demographic pattern appropriately reflects the adult population of the San Francisco Bay Area.

3.2. CD8+ T-cell analysis results

Tissue sections were subsequently evaluated for the degree of CD8+ T-cell infiltrate and analyzed for correlation between survival and CD8+ T-cell infiltration (Fig. 2). Tumors from long-term survivors were more likely than those from short-term survivors to have intermediate or extensive T-cell infiltrates compared to focal or rare infiltrates (38% for long-term; 20% for short-term survivors, $p = 0.06$). Our analysis did not note any significant differences between the invasive margin of the tumor and more central areas. Tumor heterogeneity was not suggestive or correlated with an improved outcome.

3.3. Multivariate Regression Analysis

In the multivariate regression analysis, utilizing the logistic regression model, the association between long-term survival and increased CD8+ T-cell infiltrate was the most significant beneficial risk factor for Caucasian women ($p < 0.006$). Thus, prolific CD8+ T-cell infiltrate appears to correlate with partitioned long-term survival in newly diagnosed GBM patients.

4 Discussion

Tumor-related antigens can be recognized by cytotoxic CD8+ T-cells in the context of major histocompatibility complex (MHC) class I expressing tumors [16-18]. In the present study,

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we analyzed prolific CD8+ T-cell infiltration within newly diagnosed glioma neoplasms to demonstrate that increased CD8+ T-cell infiltration is an associated prognostic factor for improved long-term survival. Our findings are one of the largest analysis reported to date that characterize the results specific for CD8+ T-cells in the setting of newly diagnosed glioblastoma.

Additional cancer studies into the presence of infiltrating T-cells have been investigated in several neoplasms such as melanoma [19-21], ovarian cancer [22-24], and colorectal cancer [25-28], but unlike in these lesions, the clinical outcome of glioma patients with CD8+ T-cell infiltrate has remained uncertain [1]. Early studies into T-cell infiltrates into glioma indicated that there was a subset of gliomas with T-cell infiltrate, but no clinical correlation was observed in these studies [29,30]. These early investigations did establish, however, that 28% to 60% of gliomas demonstrated tumor infiltration with lymphocytes. Several studies since then have confirmed the varying degrees of lymphocytic infiltraion in human gliomas with differing infiltration rates [31-34]. Other studies of glioma lymphocyte infiltration have characterized the mix of lymphocyte-infiltrating gliomas as a mixture of different lymphocytes including CD4+ and CD8+ cells [35,36]. Although these papers report varying presence and degree of lymphocytic infiltration, they confirm the presence of lymphocytic infiltration rather than any correlation with long-term clinical outcome.

Several studies have evaluated the relationship between infiltrating lymphocytes and clinical outcome. Brooks et al. report that 45% of gliomas expressed lymphocyte infiltration and that these findings correlated with survival for the patients [37]. Several other studies have suggested a positive correlation between lymphocyte infiltration and survival, but these studies did not evaluate the T-cell subset in these infiltrating lymphocytes [38,39]. Conversely Schiffer et al. in a study of 324 patients found no correlation with infiltrating lymphocytes and clinical outcomes [40]. In a study investigating the lymphocyte subsets, Rossi et al. found no correlation between clinical survival and CD8+ T-cell infiltration [41]. Furthermore, Safdari et al. in their study of 342 glioma patients report a negative correlation between infiltrating lymphocytes and clinical survival, as those patients with infiltrating lymphocytes had shorter survival times [42]. These studies represent disconcordant results and do not support a clear relationship between lymphocytes and clinical survival.

This poorly characterized association between lymphocytes and clinical outcome may be due to the lack of lymphocyte subset classification such as CD4, CD8 and regulatory T-cells performed in these reports. Recent studies have demonstrated the presence of a T-cell subset, the regulatory T-cells, which can be found in gliomas exerting an overall immunosuppressive and anergic response [43-47]. Furthermore, due to tumor heterogeneity, differing methodologies, short length of follow-up, and inconsistent sampling, these reports investigating lymphocyte infiltration may contain ambiguity in their clinical correlation.

Our study of newly diagnosed gliomas represents one of the largest studies reported to date that specifically evaluates the subset of CD8+ T-cell infiltration in newly diagnosed human glioblastomas, utilizing a systematically consistent analysis of newly diagnosed glioblastomas with consistent pathologic review and sampling. Our results here show that for patients who have intermediate or extensive CD8+ T-cell infiltrate at the time of new diagnosis with glioma were more likely to have long-term survival than patients with rare or focal CD8+ T-cell infiltrates ($p = 0.06$). Our data analysis is further bolstered by our specific comparison of patients with CD8+ infiltrates against patients who did not have CD8+ infiltrate. Furthermore, our analysis comprehensively evaluated long-term survival of patients, and suggests that patients surviving for longer than 1 year had an increased statistical association with infiltrating CD8+ T-cells. Our analysis also did not reveal significant differences inT-cell densities in infiltrative glioma tissues nor any change in

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CD8+ T-cell densities in the adjacent neuronal tissues. This suggests that perhaps the infiltrative nature of these CD8+ T-cells may have a critical role in elucidating the mechanism for this positive correlation. Future studies evaluating the immune cell density in adjacent normal brain tissue may reveal important characteristics of the immune environment of adjacent normal brain tissue surrounding glioblastomas. Our evaluation of newly diagnosed glioblastoma in this adult population suggests a significant prognostic factor for newly diagnosed glioblastoma prior to any treatment effect, and demonstrates a clear immunohistochemically identified difference in the long-term survivors compared to the short-term survivors regarding CD8+ T-cell infiltrate.

A limitation of these results is that these data represent a retrospective neuropathologic review establishing the correlation specifically between CD8+ T-cell infiltrates and long-term survival, but this neither demonstrates causation nor provides a clear mechanism [18]. Hence, these data cannot provide an actuarial or time-dependant analysis of T-cell infiltrate if it varied at different time points in the disease. It was our aim that the large nature of this analysis, utilizing a specific subset characterization of the lymphocyte infiltration, and the rigorous standardized adherence to WHO histopathologic diagnosis with central neuropathologic review would help mitigate these retrospective limitations. Future histopathologic and prospective studies may include monitoring the activation of T-cell infiltrates using specific markers, and the molecular profiling of known immunosuppressive pathways of glioma tissue in individual patients [48]. This final limitation suggests that prospective analysis of T-cell infiltrates may be informative with further CD4+ T-cell or regulatory T-cell characterization and other immune cell infiltrates that could not be performed in a retrospective analysis.

The demonstrated relationship between the degree of CD8+ T-cell infiltrates and clinical outcomes continues to provide support and promise for T-cell based immunotherapies that are currently in clinical investigation [16,49-51]. In the setting of ongoing clinical immunotherapuetics, modifications of the infiltrating T-cell populations are observed alongside successful responses [52,53]. Thus, continuing to elucidate the intricacies of the intimate interaction between glioma and infiltrating T-cells will be necessary for optimizing immunotherapeutic treatment modalities.

Conclusion

Here we report one of the largest series to date specifically evaluating CD8+ T-cell infiltration with long-term clinical survival in newly diagnosed human glioblastoma that systematically analyzes CD8+ infiltrate in newly diagnosed glioma with central neuropathologic evaluation. Our data here indicate that CD8+ T-cell infiltrate in patients with newly diagnosed glioblastoma is significantly associated with prolonged clinical outcome. This association appears to be most significant in women of Caucasian ethnicity. Prolific CD8+ T-cell infiltration may prove to be an important clinical prognostic factor in the treatment of newly diagnosed human glioblastomas.

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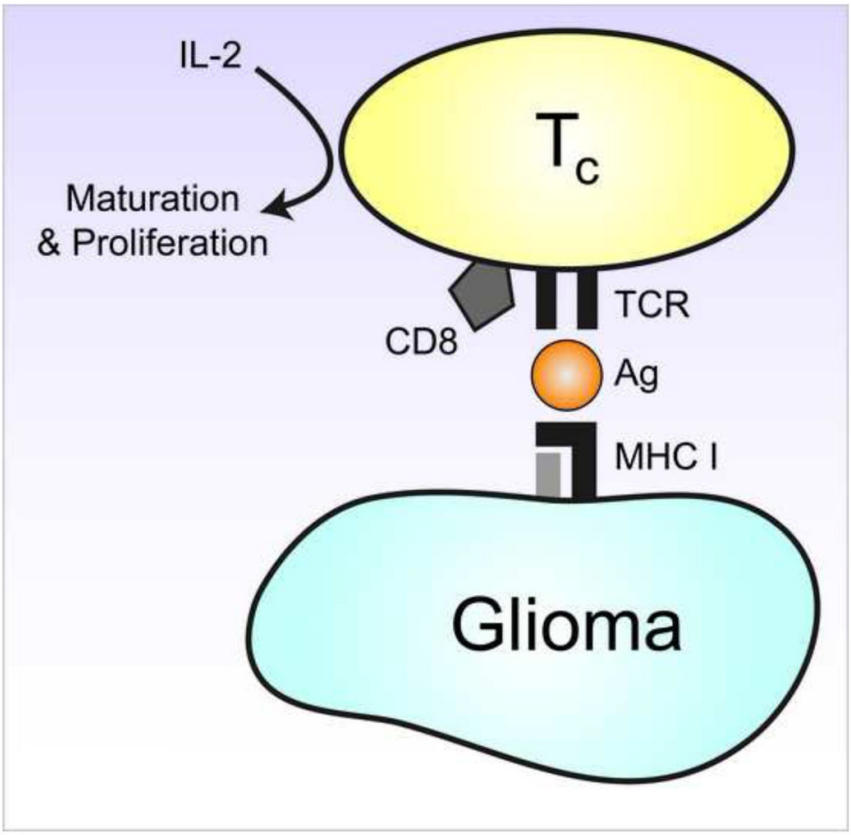

Figure 1.
Diagram of cytotoxic CD8 T-cells showing the major histocompatibility complex (MHC) I=mediated antigen-dependant T-cell mechanism for cell killing and antigen recognition.

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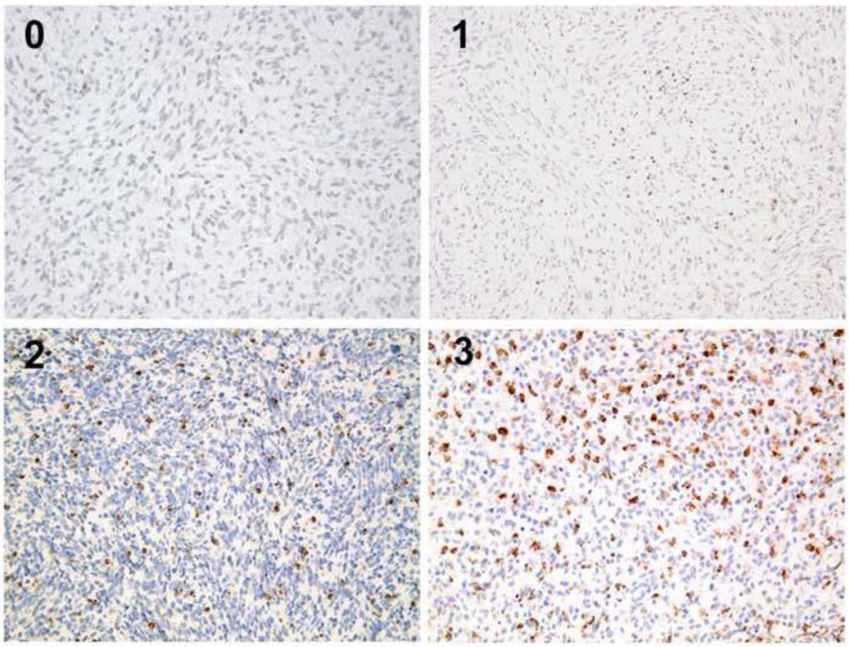

Figure 2.
Glioma tumor cells under light microscopy with varying levels of cytotoxic T-cell (CD8+ T-cell) infiltrate with human CD8 antibody and eosin counterstain (magnification ×20).

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Table 1

Comparison of patients with glioblastoma with CD8+ T-cell infiltrate results compared to those on whom staining could not be performed

	Poor long-term survival group		Extended long-term survival group	
	With results	Without staining	With results	Without staining
	n **= 43**	***n*** **= 30**	***n*** **= 65**	***n*** **= 21**
Mean age (yrs)	65.2	71.6	54.3	54
Median age (yrs)	67	74.5	54	53
Mean survival (days)	49.2	43.1	799.2	604
% Male	48.8	63.3	63.1	76.2
% White	93	80	83.1	81