REVIEW

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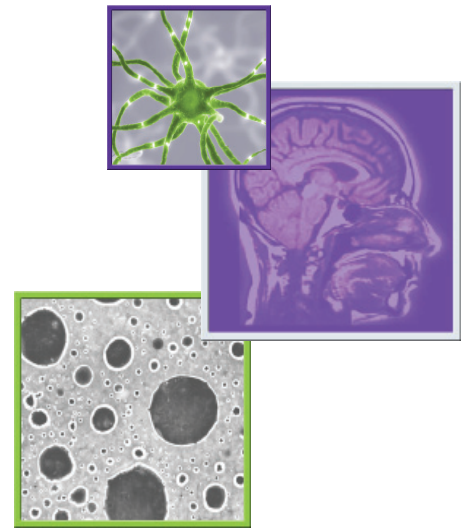
Severity, etiology and possible consequences of treatment-related lymphopenia in patients with newly diagnosed high-grade gliomas



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Practice Points

- Standard therapy for malignant glioma currently includes corticosteroids, radiation therapy and temozolomide, all of which are potentially toxic to lymphocytes.
- Over 40% of patients treated with this standard regimen develop grade 3 or greater lymphopenia, and opportunistic infections are common without the use of prophylactic antibiotics.
- Lymphopenia is also associated with decreased disease-specific survival in malignant glioma patients.
- Lymphocytes are key effector cells in the immune response to cancer, and depleting their numbers may reduce the efficacy of immunotherapeutic strategies.
- Further research is needed to determine whether treatments that increase the number of circulating lymphocytes can improve survival in this group of patients.

SUMMARY Lymphopenia is a common consequence of therapy for malignant glioma. Current standard therapy includes corticosteroids, temozolomide and radiation therapy, all of which are toxic to lymphocytes. The resulting immunosuppression has serious clinical consequences. Decreased lymphocyte counts can result in opportunistic infections, decreased efficacy of immunotherapy and reduced overall survival. The exact mechanisms underlying the association between decreased survival and lymphopenia in malignant glioma patients are unclear. However, as lymphocytes are key effector cells in the immune response to cancer, it is likely that depleting their numbers renders the immune system less effective at eliminating malignant cells. Currently, no strategies exist for the prevention or reversal of treatment-related immunosuppression in malignant glioma patients, although there are several promising theoretical approaches. This article reviews the current state of knowledge regarding the severity, etiology and possible consequences of treatment-related lymphopenia in patients with malignant glioma.

Current standard of care

During the past decade, considerable progress has been made in the treatment of patients with high-grade gliomas. Although these tumors remain surgically incurable due to their diffusely invasive nature, aggressive surgical resection is currently associated with less morbidity, allowing patients to begin adjuvant treatment with

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reduced tumor volumes and better performance status. Adjuvant chemotherapy and radiation prolong survival, but do not offer a cure. These patients also routinely receive glucocorticoids to control peritumoral brain edema, which can be caused by the tumor itself or can be a result of treatment. Many different radiation techniques have been explored, but none (including proton therapy, stereotactic radiosurgical boost and hypofractionated treatment) have been shown to be superior to the standard of 60 Gy external-beam radiation given in 30 fractions over 6 weeks. Chemotherapy also improves survival in patients with newly diagnosed glioblastoma and anaplastic oligodendroglioma. The current standard for patients with glioblastoma includes radiation therapy to enhancing and nonenhancing tumor identified on an MRI scan plus an appropriate margin. Temozolomide is given at a dose of 75 mg/m^2 daily for the 6 weeks of radiation; 6 more months of temozolomide alone are then given at a dose of 150–200 mg/m^2 for 5 consecutive days per month. This regimen improves median survival from 12 to 14.6 months, survival at 2 years from 10 to 26%, and survival at 5 years from 2 to 10% [1]. Studies evaluating the efficacy of procarbazine, lomustine and vincristine combined with radiation for anaplastic oligodendroglioma were initiated in the 1980s. Survival data from these studies have been recently reported and document a striking improvement when compared with radiation alone (7 vs 14 years) [2]. Despite the positive results of these trials, no curative therapy for malignant glioma has yet been developed and new therapies are sorely needed.

Despite these advances, the median survival of patients with glioblastoma remains short. Consequently, the development and testing of novel agents and treatment approaches are high priorities for clinicians, researchers and pharmaceutical companies. Angiogenesis inhibitors have been the subject of considerable interest in recent years. Bevacizumab, a VEGF inhibitor, is currently US FDA-approved in the USA for the treatment of recurrent glioblastoma based on its ability to restore blood–brain barrier integrity and improve clinical status. However, a survival benefit associated with this agent has yet to be documented. Bevacizumab and cilengitide (another angiogenesis inhibitor) are currently being evaluated in cooperative group clinical trials. The results of randomized prospective studies examining the efficacy of these agents in patients with newly diagnosed glioblastoma should be available in 2013 and will provide insight into the effect of inhibiting angiogenesis in glioblastoma.

There has also been considerable enthusiasm for immunotherapy to complement standard radiation and chemotherapy. This is exemplified by the development of vaccines for patients with high-grade gliomas that are usually administered after completion of radiation [3]. Interactions among radiation therapy, chemotherapy, immunosuppression (both treatment- and tumor-induced), vaccines and other immunotherapy strategies such as adoptive T-cell transfer remain highly complex and poorly understood [4–6]. In addition, the Radiation Therapy Oncology Group will soon be starting a large prospective randomized study of ipilimumab, a CTLA-4-targeted agent that modulates lymphocyte function, with radiation and temozolomide in patients with newly diagnosed glioblastoma. The efficacy of these immunomodulatory strategies could be significantly affected in immunocompromised patients.

Relevance of the lymphocyte population to the control of cancer

Lymphocytes comprise about 30% of the normal human white blood cell population and are essential effector cells in the immune response to cancer [6]. Lymphocytes arise from progenitor cells in the bone marrow. The T lymphocytes mature in the thymus, and B lymphocytes mature in the bone marrow. After maturation is complete, lymphocytes circulate in tissues, lymph nodes and the bloodstream. The human immune system has several mechanisms for identifying cancer cells and removing them from the body, most of which involve lymphocytes. Natural killer cells can be activated by multiple stimuli, including the lack of MHC-I expression, which is a common property of cancer cells. T cells can also become activated in response to malignant cells, although this process is often complicated by the development of T-cell tolerance to the malignant cells [7]. In turn, cancer cells appear to be capable of a host of immunosuppressive responses including direct suppression of T-cell function [6].

The presence of adequate populations of circulating lymphocytes appears to be correlated with the efficacy of pharmacologic antitumor immune modulators. For example, the novel agent ipilimumab, which inhibits the activity

of the T-cell-regulatory receptor CTLA-4, has demonstrable activity against melanoma and is the subject of an upcoming large Phase III trial by the Radiation Therapy Oncology Group in glioblastoma. Its efficacy appears to be dependent on the circulating lymphocyte count [8]. Ipilimumab also appears to have limited efficacy in patients receiving glucocorticoids, which are known to be immunosuppressive. Additionally, cancer vaccines, including both peptide and dendritic cell vaccines, rely on lymphocytes to be effective. The recently approved prototypic cancer vaccine sipuleucel-T (Provenge®) is created by isolating mononuclear white blood cells (lymphocytes and monocytes) from an individual patient's serum by plasmapheresis [9,10]. Antigen-presenting cells derived from this cell collection are cultured with a chimeric antigen that consists of prostatic acid-phosphatase (PAP) fused to the immune stimulant GM-CSF. These cells, which are primed to present PAP antigen to resident T cells, are then reinfused into the patient. Theoretically, the patient's own T cells should then 'learn' to attack cells that express the PAP antigen (i.e., prostate cancer cells).

Observations regarding treatment-related lymphopenia in malignant glioma patients

Pretreatment lymphopenia is a known independent prognostic factor in a variety of solid tumors, including rectal cancer, cervical cancer and metastatic breast cancer [11–13]. However, a second lymphopenic syndrome, referred to as treatment-related lymphopenia, has recently been identified and shown to have adverse outcomes. This syndrome is characterized by patients with normal pretreatment lymphocyte counts who develop severe lymphopenia shortly after receiving radiation and chemotherapy. It first became apparent when opportunistic infections, including *Pneumocystis jiroveci* pneumonia, cytomegalovirus and disseminated *Strongyloides stercoralis*, were observed among patients treated with radiation and corticosteroids alone. These patients were subsequently found to have significantly depressed CD4 counts [14–16]. The first prospective study to demonstrate the potential importance of this syndrome was conducted by the NIH-funded Adult Brain Tumor Consortium. The research began in 2004 when temozolomide, a known lymphotoxin, became part of standard therapy for glioblastoma along with radiation and glucocorticoids. Concerned that additional serious opportunistic infections might occur with the addition of temozolomide, the Adult Brain Tumor Consortium prospectively followed serial lymphocyte and CD4 counts in 98 newly diagnosed patients with malignant gliomas receiving radiation, temozolomide and glucocorticoids and correlated the results with adverse outcomes [17]. This study demonstrated a median reduction in CD4 count of 69%, and CD4 counts dropped below 200 cells/mm^3 in approximately 45% of patients. Multivariate analysis demonstrated that severe lymphopenia was an independent predictor of poor survival. Deaths were secondary to progressive disease and not to opportunistic infections. Emerging data from patients with pancreatic cancer suggest that a similar relationship between post-treatment lymphopenia and decreased overall survival also exists in this patient population [18].

Etiology of lymphopenia in brain tumor patients

Lymphopenia is a common side effect of standard malignant glioma treatment, as all of the standard treatments are lymphotoxic. The majority of brain tumor patients require treatment with corticosteroids at some point in the course of disease owing to the presence of peritumoral cerebral edema. Corticosteroids are extremely effective at relieving cerebral edema but are also potent immunosuppressants. These drugs are commonly used to treat inflammatory/autoimmune diseases and to induce immunosuppression in organ transplant recipients. Corticosteroids cause lymphopenia in approximately 40% of individuals receiving these drugs for at least 1 month [19]. A recent study of 26 glioblastoma multiforme patients showed that individuals treated with corticosteroids demonstrated T-cell-specific lymphopenia and an increase in abnormal monocytes, which were less likely to differentiate into dendritic cells and had a decreased capability to stimulate T-cell proliferation [20]. The mechanisms underlying corticosteroid-induced immunosuppression are poorly understood but appear to involve a variety of effects on the immune system, including inhibition of cytokine production, induction of apoptosis in mature T lymphocytes and interference with adhesion of antigen-presenting cells [21].

Temozolomide is an orally bioavailable alkylating agent that can penetrate the blood–brain barrier. It is the only first-line chemotherapy agent approved for patients with glioblastoma.

This agent is also often used off-label for patients with grade 3 astrocytoma and oligodendroglioma. Temozolomide was established as the standard adjuvant chemotherapy for patients with glioblastoma by a landmark European Organisation for Research and Treatment of Cancer Phase III study that demonstrated a doubling of the 2-year survival rate (from 10 to 26%) when this drug was used in conjunction with radiation [1]. Fatigue, mild nausea and hematologic toxicity are the most common adverse effects reported with the use of temozolomide. Among patients receiving temozolomide, the risk of neutropenia is low (<10%), but grade 3–4 reductions in lymphocyte and platelet counts are fairly common. In a study of 97 patients receiving extended-schedule single-agent temozolomide for metastatic melanoma (over 90% of whom had normal lymphocyte counts before beginning therapy), 60% developed lymphocyte counts of <800/µl and 33% developed lymphocyte counts <500/µl after 3 months of treatment [22]. In patients receiving temozolomide for >200 days, the estimated incidence of lymphopenia was 77%. Lymphopenia was also prolonged, with the majority of patients having persistently depressed lymphocyte counts at least 2 months after stopping temozolomide. Temozolomide appeared to be selectively toxic to $CD4^+$ lymphocytes, with a median nadir $CD4^+$ lymphocyte count of 150/µl among the patients on this study. Selective toxicity to CD4 cells is probably responsible for the opportunistic infections seen among patients receiving temozolomide. In the study described above, five patients developed *P. jiroveci* pneumonia, one developed aspergillosis and 21 had infections such as herpes zoster that were considered indicative of T-cell dysfunction but not strictly opportunistic. Temozolomide preferentially exerts its lymphotoxic effect on dividing lymphocytes, and its effects appear to be mediated at low doses by *O*-methylguanine [6] and at higher doses by other mechanisms of other DNA damage [23].

Radiation can be an effective therapy for brain tumors, and nearly all patients with malignant glioma receive radiotherapy at some point in the course of their illness. Radiation-related lymphopenia occurs regardless of whether patients also receive steroids or chemotherapy. This was first described in the early 20th century and has since been documented following focal radiotherapy in many different anatomic sites. The first large study evaluating long-term lymphocyte counts among women who received radiation for breast cancer was published in 1970 [24]. This demonstrated significantly decreased lymphocyte counts, even years after treatment, among women who received radiotherapy for breast cancer. At the time, it was hypothesized that thymic irradiation was responsible for lymphopenia in these patients, as targeting the internal mammary lymph nodes resulted in fairly high doses to the anterior mediastinum and thymus. This hypothesis was later disproved by another study that compared lymphocyte counts in patients receiving pelvic irradiation with patients receiving thoracic irradiation [25]. Lymphocyte counts were even lower in the pelvic irradiation group, thereby ruling out thymic irradiation as the cause of lymphopenia. Irradiation of the bone marrow has been postulated as a possible cause of radiation-induced lymphopenia, but this mechanism is unlikely to be the sole cause as radiation causes lymphopenia even when sites that contain no marrow, such as the brain, are treated.

Another possibility is that irradiation of circulating blood is responsible for the development of radiation-induced lymphopenia. This hypothesis is supported by a variety of clinical observations. In 1978, MacLennan *et al.* reported a series of children with acute lymphocytic leukemia who received prophylactic cranial radiation under the British Medical Research Council UKALL Protocols II and III [26]. The total prescribed dose of 24 Gy was specified by the protocol, but participating centers were free to choose how many fractions would be used to deliver the dose. The number of fractions varied from five to 15, and the investigators plotted the consequent decrease in the lymphocyte count in terms of the number of treatments received. They demonstrated an inverse relationship between the number of fractions and the lymphocyte count, with each additional fraction reducing the lymphocyte count by 5–6%. These data strongly implicate dose received by circulating blood as a cause of lymphopenia. Not only was there no exposure of bone marrow or thymic tissue to radiation in these patients, but with increasing number of fractions administered, time spent under the beam increases and circulating blood is exposed to more radiation. Furthermore, irradiation of blood via a radioactive source placed within a dialysis unit can induce a 60% reduction in the lymphocyte count that persists for at least 1 year [27]. The so-called 'extracorporeal irradiation of blood' was so effective at immunosuppression

that it was used to prevent graft rejection in patients undergoing renal transplant before the advent of immunosuppressive drugs. Radiation may also affect the immune system in more subtle ways, such as by inducing the production of lymphotoxic cytokines or suppressing the production of cytokines that stimulate lymphocyte proliferation. Much more research in this area is needed before any definite conclusions can be reached regarding how radiation affects cytokine levels in brain tumor patients.

Conclusion & future perspective

Investigating the role of treatment-related lymphopenia in patients with malignant glioma is an important area for research. Because the degree of treatment-related lymphopenia is associated with survival, a better understanding of this side effect is clearly needed. It is currently unclear whether decreased survival in patients with treatment-related lymphopenia is a causative relationship or simply an association. The exact mechanisms underlying the relationship between lymphopenia and decreased survival in these patients are likewise unknown. Nevertheless, the hypothesis that decreasing the number of circulating lymphocytes can depress the immune response to cancer (in which lymphocytes are crucial effector cells) is an attractive one. Probably the only way to demonstrate causality is to perform interventions that increase the lymphocyte count to determine if such treatments can improve outcomes. Because lymphocytes are not typically considered a tissue at risk during cancer treatment, no attempts have been made to spare the lymphocyte compartment from damage induced by cancer therapy. Several strategies could be used to spare lymphocytes during radiation treatment. Hypofractionated treatment plans (i.e., delivering the same biologically equivalent dose in fewer fractions) or smaller target volumes may be able to spare circulating lymphocytes. Unfortunately, not all patients have small tumors, and such planning strategies will not be applicable in a significant subset of patients with malignant glioma. In terms of chemotherapy, identifying the lowest effective dose and shortest effective duration of temozolomide treatment could potentially spare patients from immunosuppression or allow a more rapid recovery of lymphocyte counts. Bevacizumab, like steroids, can significantly reduce cerebral edema. However, bevacizumab does not cause immunosuppression and may represent an immune-sparing alternative to corticosteroids. An alternative approach is immune reconstitution following therapy using either cytokine therapies or reinfused autologous white cell transfusions.

Treatment-related lymphopenia in patients with malignant glioma occurs frequently and is often severe. It is associated with significant adverse clinical outcomes, including opportunistic infections and poorer disease-specific survival. It is possible that a subset of patients who are predisposed to poor outcomes from their tumor also possess some other currently unrecognized characteristic that renders their lymphocytes more susceptible to treatment-induced toxicity. However, it is clear that lymphocytes should be considered a tissue at risk during chemotherapy and radiation therapy for patients with malignant glioma. Future clinical trials and laboratory investigations are needed to shed more light on the prevention of lymphopenia and the restoration of immune function in brain tumor patients.

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