

# Immunopathology of Central Nervous System Tumors

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## Abstract

The central nervous system (CNS) is characterized by unique immune biology. Distinct mechanisms of CNS immune surveillance and activation have important implications in tumor development as CNS tumors are known to evade anti-tumoral immunity, and may also contribute to immunosuppression. Multiple cell-surface and secreted mediators, expressed in both CNS tumor cells and responding immune cells, have been shown to influence the immune response to CNS tumors. In this review we provide an overview of CNS tumor immune escape and immunosuppression, highlighting the cellular and molecular features associated with both CNS tumors cells and responding immune cells. In this context, we discuss of the role of the M1 and M2 tumor associated macrophage phenotypes, myeloid derived suppressor cells, regulatory T-cells, as well as many immunomodulatory cytokines. Additionally, recent insights into the STAT-3 intracellular signaling pathway and the presence of active human CMV infection in the context of CNS tumor development are discussed.

**Keywords:** Immune Biology; CNS Tumor; Immunotherapy; Glioblastoma Multiforme; STAT3; Regulatory T-Cells

## Introduction

Cancers of the central nervous system (CNS) are unique in their interaction with the immune system throughout development and progression. Advances in identifying common themes of altered immune biology across many cancers, and the recent successes of immune based therapies for some solid cancers, together have invigorated an interest in the complex interplay between CNS tumors and the immune response. Insights in the immune biology underlying CNS cancer development have already produced promising therapeutic results in preclinical and early clinical investigations, which when set against the modest effects of current CNS cancer treatment and the dismal prognosis most of these patients face, stir excitement for immune-based therapy in CNS cancer.

Cancers of the brain, spinal cord, and surrounding structures are diagnosed in approximately 9-11 per 100,000 people in the United States per year, and effect an age adjusted mortality rate of 4.3 per 100,000 per year [1]. Current treatments are rarely curative, and often balance tumor control with neurological morbidity. Treatment strategies typically involve a combination of cytoreductive surgery, chemotherapy, and radiation therapy. These treatments are largely non-specific to cancer cells, and therefore are damaging to bystander neurological tissue while achieving only modest therapeutic benefits. For instance, in a recent series reviewing the treatment of Glioblastoma multiforme (GBM) with surgery, radiation therapy, and chemotherapy with temozolomide, overall 2-year survival was only 27% [2]. Figure 1 provides an example of a GBM tumor, effecting significant neurological morbidity through extensive tissue involvement. Unfortunately, the vital functions and poor resiliency of neurologic tissue, as well as the often diffusely infiltrating nature of CNS cancers, provide insurmountable limitations to current therapies.

Anti-tumor properties of the immune system are well documented and known to be dysregulated in many human cancers, including those of the CNS [3]. The ability of intrinsic host immunity to specifically target tumor cells might be superior to current non-specific and damaging therapies aimed at eradicating tumors, though the poorly understood mechanisms by which tumor cells suppress and misdirect the immune response hinder immune-based therapies. Appreciation of the distinct features of immune activation and modulation within the CNS, coupled with a clearer understanding of tumor mediated immune suppression, may form a foundation upon which beneficial and safe immune based therapies can develop.

## CNS Immune Environment

### Tumoricidal immune system

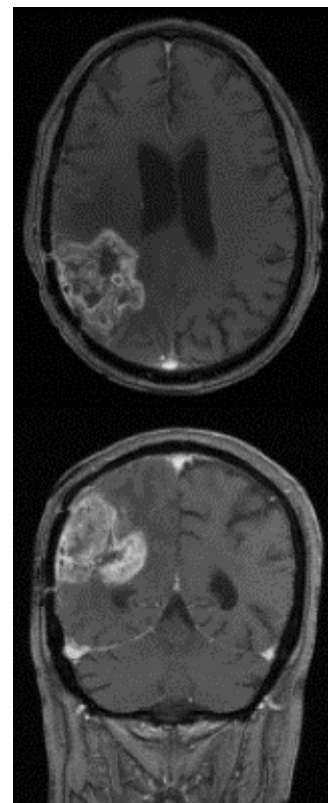
Generally, anti-tumor immune surveillance is thought to occur in three different circumstances. First, elimination of pathogens that cause chronic inflammation is believed to prevent the development of some cancers, as the inflammatory environment contains carcinogenic free radicals and genotoxic agents. A chief example lies in the affects of *Helicobacter Pylori*, a pathogen known to cause gastritis and gastric ulcer formation, and which ultimately increases a carriers risk of gastric cancer and MALT lymphoma [4]. Eradication of *H. Pylori* has been demonstrated to reduce the risk gastric cancer in symptomatic patients and has therefore become standard of practice [5]. Immune mediators of the CNS do not routinely combat inflaming pathogens, as this space is sterily barricaded with natural limits on inflammation. Still, evidence from parenchymal infection, infarction, and from autoimmune disease such as multiple sclerosis, clearly demonstrate the capacity to initiate classical inflammatory cascades within the CNS [6,7]. Second, control of oncogenic viral infections through cytotoxic lymphocytes (CTL) and natural killer (NK) cell immunity is essential to prevent viral induced genomic and transcriptional alterations that may induce neoplastic transformation. Examples of viral induced

cancers include lymphomas caused by Epstein-Barr virus [8] and cervical carcinoma caused by papilloma virus [9].

Regarding CNS tumors, infection with cytomegalovirus has been proposed to underlie the development of some gliomas considering the alarming presence of CMV antigens in glioma tissue specimens [10]. To date, this hypothesis is based exclusively on association studies and may simply reflect activation of latent infection under CNS cancer-induced immune suppression. Lastly, if the local tissue environment promotes antigen presentation and lymphocyte activation, CTLs and NK cells are capable of recognizing and eliminating tumor cells that express developmental or mutated cancer-specific antigens [3]. Accumulating evidence suggests that immune mediators capable of this final mechanism in CNS tumors are either limited in doing so, or are overcome by tumor-derived immunosuppression at some point in tumor progression. A focus of ongoing CNS cancer research will be the investigation of these limitations, and the design of therapeutic strategies, which overcome it.

### Immunocompetent Compartment of CNS

The CNS was long viewed as an “immune-privileged” site due to a perceived lack of antigen presenting cells (APCs), restriction from circulating lymphocytes and other immune mediators by the blood brain barrier (BBB), and absence of lymphatic drainage. As a result, the CNS appeared to possess little immunologic potential to resist tumor development [11]. Evidence accumulated over the last 20 years, however, has largely debunked this view of the CNS by demonstrating distinct immune activation cascades within the CNS in response to ischemia, traumatic brain injury, and autoimmune disease [7,12]. In these conditions, immune competence is dependent upon the activation of resident microglia and infiltrating macrophages capable of effective lymphocyte activation, all permissible through inducible permeability of the BBB to immune mediators [6,13]. Activated microglia has been shown to phenotypically resemble antigen-presenting cells, and subsequently is capable of activating T cell lymphocytes [14]. Following activation, CNS APCs are known to migrate throughout the CNS, and are capable of returning to the systemic circulation through drainage via perivascular Virchow-Robbin spaces and the nasal mucosa as conduits to cervical lymph nodes [15-17]. Activated and naïve T-cells responding to local chemotactic signals have been shown to traverse the BBB and engraft into sites of inflammation [18]. These activated T-cells remain in the CNS, as demonstrated in tumor extracts from multiple CNS cancers which display tumor-antigen specific CTLs and helper T -cells, and furthermore are capable of tumoricidal immune function in vitro [19-21]. Additionally, circulating CNS -antigen specific CTLs and antibodies have been isolated from the peripheral blood of patients with CNS cancer, further indicating the potential for competent tumor-specific responses within the CNS [22,23]. Despite this apparent immune-competence, recent reports have demonstrated anergy and apoptosis following TCR stimulation in CNS cancer infiltrating T- cells [20]. Moreover, immunosuppressive regulatory T-cells (Tregs) have been shown to compose a significant proportion of CNS tumor-infiltrating lymphocytes [24]. Understanding these discrepancies in lymphocyte activation versus suppression following stimulation with tumor antigens within the CNS will be imperative to the success of current CNS cancer immune based therapy research.



**Figure 1:** Magnetic Resonance Imaging (MRI) of a patient with Glioblastoma Multiforme tumor (GBM). Axial (left) and coronal (right) T1-weighted MRI images taken following the injection of gadolinium contrast clearly demonstrate a large GBM tumor in the right parietal lobe, causing significant brain edema as demonstrated by surrounding hypodense tissue signal. Current treatments involving surgery, radiation, and temozolomide chemotherapy have only modest impacts on the dismal prognosis of GBM tumors, while often effecting significant neurological morbidity. A more complete understanding of the immune biology of GBM may allow for immune-based therapies, which pose to offer more durable and less damaging therapeutic benefits.

### Immunocompetent Compartment of CNS

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## CNS Tumor Immunosuppression

Suppression of CNS immune surveillance and alternative activation of tumoricidal immune cells are both fundamental features of CNS tumor biology. Believed to be immune-competent and potentially tumoricidal, suppression of the CNS immune response by tumor cells presents the major barrier to our intrinsic ability to resist CNS cancers. Accordingly, insight into the mechanisms by which tumor cells disable immune activation and construct a suppressive tumor microenvironment are a major focus in design of immunotherapeutic strategies. Unfolding evidence implicates many cellular participants in a complex orchestration of suppression including tumor cells, resident microglia, peripherally invading macrophages, and lymphocytes, most notably Tregs. Interactions among these players are thought to underlie the state of generalized immunosuppression observed in many patients with CNS cancers, likely extending systemically from the potentially immunosuppressive local tumor microenvironment at the interface of the tumor and immune cells. Current efforts to map the immunosuppressive network engineered by malignant CNS cancers are built upon detailed characterizations of implicated cells in efforts to identify key mediators or pathways as targets to intervene upon in designing immune therapies.

## CNS Tumor Cells

Transformed cells are clear targets for CNS immune sentinels responding to expression of aberrant or mutated antigens, and to cell stress antigens associated with increased proliferation or stromal remodeling. Such antigens stimulate immune cells through activation of Major Histocompatibility Complex (MHC) class I and II molecules in coordination with co-stimulatory signals including B7 isoforms 1 and 2 (CD80/86) [25,26]. As a principle means of evading tumoricidal immune activation, CNS tumor cells markedly down-regulate expression of both MHC I and II proteins, as well as direct the down-regulation of co-stimulatory molecules on APCs [27]. Glioma cells, the most extensively studied of CNS cancer cells, demonstrate an inverse correlation between extent of MHC molecule down-regulation and tumor infiltration by lymphocytes. Furthermore, extent of MHC

molecule down-regulation was also shown to inversely correlated with tumor grade [25,28], likely reflecting the affect this mechanism has on tumor evasion of immune targeting. Additionally, through secreted mediators glioma cells have been shown to direct the down-regulation of co-stimulatory molecules B7-1 and B7-2 on APCs, most notably tumor associated macrophages (TAM), removing a necessary signal for proper T-cell activation [28,29]. Glioma cell derived mediators are believed to direct the expression of the potentially immunosuppressive co-stimulatory molecule homologue B7-H1 [30], expression of which is normally limited to germinal centers at the end of immune responses. B7-H1 expression has been demonstrated on both glioma cells themselves as well as on TAMs, and functions to induce apoptosis in activated T cells, as well as to stimulate the proliferation of immunosuppressive Tregs.

Expression of secreted immunomodulatory mediators and cell-surface proteins by glioma cells also contribute to immunosuppression and tumor propagation. Immunosuppressive mediators known to derive from glioma cells include transforming growth factor beta (TGF-B), prostaglandin E2 (PGE2), Fas ligand (FasL), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and the immunomodulatory cytokines IL-4, IL-6, and IL-10 [26]. Shown to potentially stimulate glioma cell proliferation, TGF-B is also known to inhibit development and activation of APCs, repress activation of NKs, and prevent the activation and differentiation of CTL [31]. T-cell activation and proliferation are suppressed in the presence of PGE2, which has also been demonstrated to induce the production of immune-regulatory Tregs and to promote glioma cell proliferation through induction of protein kinase A (PKA) [32]. One of the principle mediators of programmed cell death in a variety of cells types is the cell surface protein FasL, which has been detected on the surface of many CNS cancer cell types, as well as in multiple CNS cancer cell lines [33,34]. Both microglia and T-cells express the FasL receptor, Fas, and therefore may be susceptible to the death signal provided by FasL expressed on tumor cells. Indeed, multiple studies have demonstrated that FasL was responsible for the death of T lymphocytes when co-cultured with glioma cells in vitro, and that the down-regulation of FasL on tumor cells enhances tumor infiltration by T-cells, reducing tumor growth in vivo [35]. Unrestrained expression of VEGF by CNS tumor cells is known to drive angiogenesis and to provide a potent chemotactic signal to monocytes. In conjunction with EGF and TGF-B, VEGF is also believed to stimulate tumor cell proliferation [36]. Increased expression of the immunomodulatory cytokines IL-4, IL-6, and IL-10 has been demonstrated in multiple CNS cancers, most notably GBM cells. These cytokines limit inflammation, reduce immune activation, and drive the expression of immunosuppressive mediator such as TGF-B and PGE2 [37].

Recently, expression of indoleamine 2,3 dioxygenase (IDO) by glioma cells has been implicated in the recruitment of immunosuppressive Tregs, and the subsequent ablation of anti-tumoral immunity [38]. A series of in vivo experiments showed that IDO-derived Treg tumor infiltration led to a decrease of CTL tumor infiltration, and in contrast, IDO silencing on tumor cells led to an increase in CTL infiltration corresponding with an increase in overall survival for mice bearing glioma xenografts. Interestingly, the authors further demonstrated that tumor-cell specific expression of IDO, rather than peripheral expression of this enzyme is critical for maintaining this immunosuppressive state [38]. IDO might have a clinical and translational therapeutic potential, as its expression correlates with tumor grade and has a negative impact on overall survival for patients with gliomas [39].



Ongoing efforts to functionally characterize CNS tumor cells continue to implicate cell-cell and cell secreted mediator interactions in addition to those discussed above. Altogether, these interactions are hypothesized to function in autocrine and paracrine signaling loops that construct a complex local microenvironment involving tumor and immune cells, which is both potentially immunosuppressive and tumorigenic [26].

## Tumor Associated Macrophages

Tumor associated macrophages (TAMs) are the predominant infiltrating immune cells in malignant glioma, and can account for up to 40% of the tumor cell mass [28]. Phenotypically indistinguishable following activation, TAMs are derived from both resident CNS microglia and from bone marrow derived mononuclear cells known to colonize the CNS under pathological conditions [40]. The relative abundance of TAMs compared to lymphocytes in CNS tumors has directed considerable attention to this population, postulating that, under the influence of glioma cells, TAMs play a major role in the creation of the immunosuppressive and tumor promoting local tumor microenvironment [41,42].

Considerable efforts to characterize TAMs in glioma have led to delineation between classically activated inflammatory M1-type macrophages with tumoricidal potential from alternatively activated immunosuppressive M2-type macrophages, thought to predominate in CNS tumor microenvironment. Classically activated M1-type macrophages participate in the coordinated response to immunogenic antigens primarily through production of pro-inflammatory and tumoricidal mediators such as NO, TNF- $\alpha$ , IL-1B, and IL-12, up-regulation of surface molecules necessary for antigen presentation, and an enhanced ability to phagocytose pathogenic material [20,26]. Conversely, alternatively activated M2-type macrophages do not secrete the pro-inflammatory mediators NO, IL-1B or TNF- $\alpha$ , and are believed to exert immune-modulation through multiple mechanisms including secretion of potent immunosuppressive and tumorigenic mediators including IL-10, IL-6 and TGF- $\beta$ , down-regulation of MHC and co-stimulatory molecules, decreased phagocytic capability, and up-regulation of cell surface antigens FasL and B7-H1 [43,44]. The alternate M2 macrophage phenotype therefore does not contribute to anti-tumor immunity, and appears rather to support tumor progression through expression of both immune dampening and tumor promoting mediators. Reduced generation of tumoricidal mediators such as NO, IL-1B and TNF- $\alpha$ , and the expression of both FasL and B7-H1 lead to the induction of energy and apoptosis in effector T-cells, which express Fas. Thus, TAMs appear to play a role in tumor-induced immunosuppression. See Figure 2 for a summary of TAM phenotypes.

Within CNS tumors, engrafted TAMs overwhelmingly demonstrate the M2 phenotype [39]. Furthermore, the presence of M2 type TAMs appears to correlate with tumor grade. A recent investigation demonstrated increased expression of the alternatively activated M2 markers CD163 and CD204 by TAMs in WHO grade IV gliomas, as compared to those from WHO grades II and III gliomas [45]. The perverse polarization of TAM precursors to the alternative M2 state, both in resident microglia and peripheral derived monocytes, is generally believed to occur as these cells encounter the myriad growth factors and surface antigens of the tumor microenvironment established by tumor cells. Among the factors implicated in the recruitment and M2 polarization of monocytes by CNS tumor cells, monocyte chemoattractant proteins 1 (MCP-1/CCL-2), and monocyte

colony stimulating factor (M-CSF) and believed to drive local recruitment and proliferation of TAM precursors [46,47], while TGF- $\beta$ , IL-4, IL-10, IL-13 together orchestrate polarization to the alternative M2 phenotype [48,49]. Importantly, this polarization toward a M2 TAM phenotype occurs in the absence of IFN- $\gamma$ , a potent driver of the classical M1 phenotype [50]. The absence of IFN- $\gamma$  is likely due to the suppressed activation of its principle source, activated type 1 T helper cells, discussed below.

A recent investigation into TAM associated immunosuppression in CNS tumors utilized high-throughput gene expression profiling on stimulated monocytes in the presence or absence of GBM tumor cells. This analysis identified caveolin-1 (CAV1) as significantly upregulated in both stimulated monocytes cultured in the presence of GBM cells, as well as in TAMs isolated from ex-vivo GBM tumor specimens following surgical resection [51]. CAV1 is known to alter inflammation through multiple immunosuppressive and anti-inflammatory mechanisms [52,53], and its expression has been demonstrated in many different immune cell types [54,55]. Accordingly, upregulation of CAV1 in GBM TAMs may directly reflect tumor-derived immunosuppression, acting as an intracellular mediator of the alternatively activated M2 TAM phenotype. Additionally, rescue of an inflammatory myeloid phenotype may be produced by siRNA knockdown of CAV1 in monocytes co-cultured with GBM cells, as indicated by a restoration of TNF- $\alpha$  secretion [51]. This promising finding may allow for therapeutic intervention against CAV1 expression in TAMs, effecting a reduction in tumor-derived immunosuppression and a restoration of inflammatory and anti-tumoral immunity.

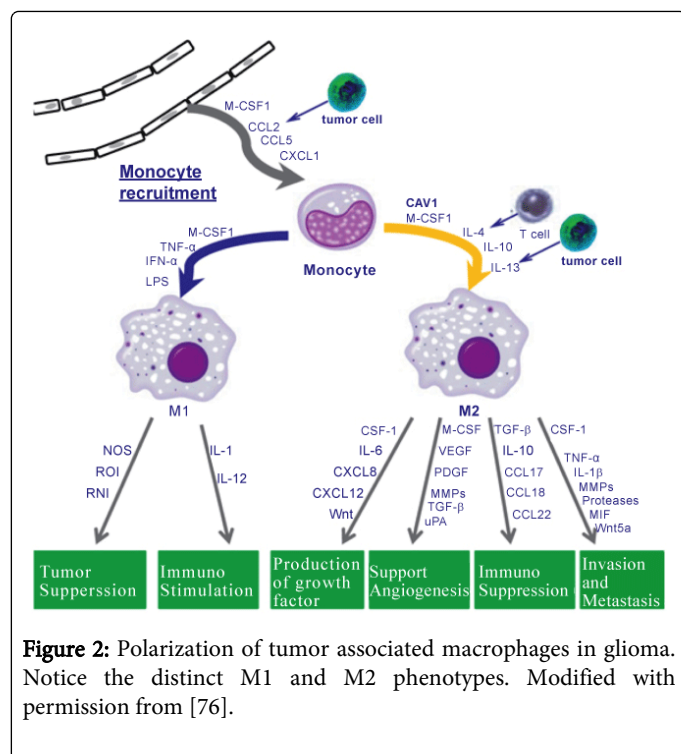
## Myeloid Derived Suppressor Cells

A refinement of the M1/M2 TAM characterization scheme describes a more heterogeneous population of myeloid-derived cells at different stages of maturation, able to suppress multiple phases of the immune response [56]. These myeloid-Derived Suppressor Cells (MDSC), have been shown to both perpetuate tumor-promoting microenvironments, as well as to distribute peripherally to hinder lymphocyte activation in immune organs. MDSC are therefore believed to contribute to the general systemic immunosuppression observed in many patients with CNS cancers [57]. Initially postulated to arise peripherally from the influence of circulating tumor derived immune factors, more recent evidence implicates a concentrated cocktail of immunomodulatory mediators and cell-cell interactions in the tumor microenvironment necessary to direct MDSC fates in myeloid precursors [57]. These observations suggest that naïve monocyte traffic to the tumor microenvironment, mature into immunosuppressive MDSCs, then redistribute systemically to effect general immunosuppression. Heterogeneous in expression profile and immunomodulatory function, MDSC present a poorly understood hurdle to remediating CNS cancer immune suppression. If indeed this phenotype is driven by tumor derived factors, as is hypothesized for the alternative M2 phenotype of TAMs, disabling the local "monocyte-educating" mechanisms of tumor cells and M2 TAMs may reduce the number and function of MDSC.

## Lymphocytes and Regulatory T-cells

T-cells provide an essential link between a pathogenic stimulus and the adaptive immune system. T-cell receptor (TCR) recognition of transformed cell antigens presented in the MHC molecules of APCs or tumor cells themselves, with the necessary co-stimulatory signals, may

result in the generation of CTL specific for tumor antigens [3,58]. Furthermore, if a tumor antigen is also recognized by B cells, activated plasma cells may produce tumor antigen specific antibodies [3]. As discussed above, this process is severely hindered in CNS cancers by the reductions in the expression in MHC and co-stimulatory molecules on both tumor cells and surrounding APCs, and by the T-cell deactivating milieu within the tumor microenvironment. Broadly, a reduction in the ratio of Th1/Th2 polarize T-cells has been demonstrated among lymphocytes cultured from patients with primary or recurrent GBM, as compare those cultured from healthy subjects and meningioma patients [59]. The innate immune system, specifically NK cells, is known to initiate deletion of T-cells with reduced expression of MHC or co-stimulatory molecules through the release of TNF- $\alpha$  and IFN- $\gamma$  [25]. This fail-safe mechanism is also disabled by the immunosuppressive milieu of the local tumor microenvironment, most notably by IL-10, and by stimulation of the NK cell inhibitory receptor KIR2DL through the ligand HLA-G, which is expressed Tregs [58]. Through these mechanism lymphocytes that are polarized to immunosuppressive phenotypes are permitted to remain within CNS tumors.



Tregs are an important lymphocyte player in CNS tumor immune biology. Commonly defined by the combined expression of CD4, CD25, and Foxp3, Tregs are T-cell lymphocytes that effect negative modulation of immune responses, and are believed to limit autoimmunity through inhibition of autoreactive effector T-cells [24]. The systemic depletion of Tregs is associated with a wide variety of autoimmune diseases, and restoration of Tregs in deficient mice has been shown to rescue crippling autoimmune conditions [60]. An increased systemic presence of Tregs is observed in many malignancies, including malignant glioma, consistent with their proposed role in suppressing the immune response to neoplastic cells [61]. Furthermore, infiltration of brain tumors by Tregs has been shown to correlate with tumor grade [62]. These observations likely reflect the significant role Tregs play as a negative modulator of

lymphocytes both locally within the tumor and peripherally in lymphoid organs, together allowing for immune evasion of tumor cells.

Investigation of the origin, recruitment, expansion, and immunomodulatory effect of Tregs in malignant brain tumors is an active area of immunology research. Naturally arising Treg (nTregs) originate in the thymus and are believed to effect peripheral tolerance by expressing TCRs for self-antigens, thereby dampening autoreactive T cells [53]. Alternatively, recent evidence has demonstrated that CD4+/Foxp3<sup>-</sup> T-cells may be converted to CD4+/Foxp3<sup>+</sup> induced Tregs (iTregs) peripherally through exposure to suboptimal TCR stimulation in the presence of high levels of TGF- $\beta$ , as is present in the tumor microenvironment [63]. Both nTreg and iTreg subtypes have been shown to infiltrate and proliferate within CNS tumors; migration occurs in response to tumor cell derived MCP-1 through it's receptor CCR4, present on the surface of Tregs and their precursors [58]. Tregs elicit immunosuppression principally through Foxp3 controlled expression of the immunosuppressive cell surface ligands glucocorticoid-induced tumor necrosis factor receptor (GITR), cytotoxic T-lymphocyte antigen (CTLA-4), and human leukocyte antigen G (HLA-G) [64]. Additionally, Tregs have been shown to contribute the immunosuppressive cytokines TGF- $\beta$  and IL-10 to the tumor microenvironment [24]. GITR is expressed in high amounts on the surface of Tregs, and has been shown to induce expression of immunosuppressive mediators in response to many ligands of the TNF family, thus providing counter-regulatory feedback diminution of TNF driven pro-inflammatory signals [65]. CTLA-4 has been long recognized as a T cell "off switch" which binds the co-stimulatory molecules B7-1 and B7-2 to deliver an inhibitory signal concomitant to the binding of a peptide presented in the MHC molecule of an APC [66]. This inhibitory signal replaces the stimulatory interaction between T cell protein CD28 and APC co-stimulatory molecules B7-1 and B7-2 to prevent activation. HLA-G on placental cells has been shown to contribute immune tolerance in pregnancy by binding the KIR2DL receptor of NK cells, blocking activation in the presence of cells lacking MHC or co-stimulatory molecules. By this mechanism Tregs are hypothesized to disable NK cell surveillance. Altogether, these immunomodulatory mediators expressed by Treg that engraft into tumors of the CNS dampen tumoricidal immunity and facilitate tumor cell immune escape.

### STAT-3 pathway

As discussed, many soluble mediators and cell surface molecules expressed by tumor cells, TAMs, and Tregs participate to establish a potentially immune disabling tumor microenvironment. Expression profiles across these various cellular players are similar, raising suspicion for unifying mediators of signal transduction or gene expression common to these shared phenotypes. Increased activation of the signal transducer and activator of transcription protein 3 (STAT-3) in glioma tumor cells and glioma TAMs provides evidence of a shared intracellular mediator of immunosuppression [67,68]. Furthermore, considering the many targets of STAT3 modulation, activation of this intracellular mediator may also augment CNS tumor angiogenesis and stromal remodeling [69]. STAT3 activation in glioma TAMs is induced downstream of many mediators known to compose the local tumor microenvironment including IL-10, IL-6, EGF, and FGF [70]. Activated STAT3 in both tumor cells and TAMs is known to effect a reduction in the expression of surface molecules necessary for antigen presentation such as MHC-II, B7-1, and B7-2, as well as to increase the expression of many M2 specific immunomodulatory

mediators including IL-10, EGF, VEGF, and various matrix metalloproteinases (MMPs) [71]. Experiments blocking the activation of STAT3 in glioma cancer stem cells (gCSC), a sub-set of relatively undifferentiated tumor cells, co-cultured with allogenic T-cell precursors demonstrate reduced Treg differentiation and reduced overall Tcell apoptosis [72]. Therefore, STAT3 may serve as a critical “molecular hub” linking multiple immunosuppressive pathways in CNS tumor cells and alternatively activated M2 type TAMs. Furthermore, STAT3 target molecules such as IL-10 and IL-6 have been shown to subsequently activate STAT3 [73], leading authors to a proposed a feed-forward mechanism of reinforced STAT3 activation, which may account for the constitutive activation of STAT3 in both glioma cells and glioma-infiltrating TAMs.

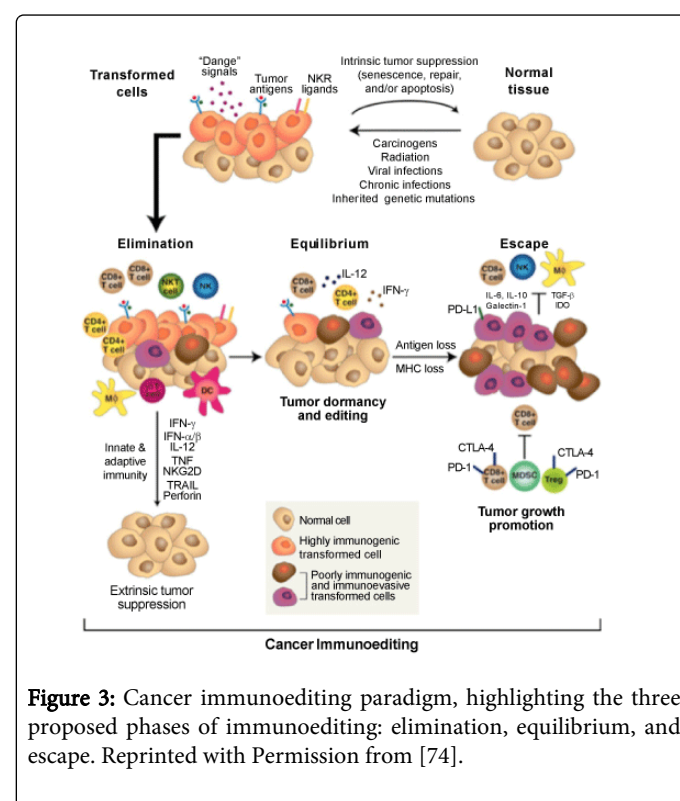
## Cytomegalovirus in Glioma

Accumulating evidence demonstrating an association between active human CMV infection and malignant glioma may provide additional insight into tumor related immune suppression. A recent investigation reported the presence of CMV associated nucleic acids and proteins in over 90% of ex vivo GBM specimens analyzed. Neither CMV associated nucleic acids or proteins were present in surrounding normal brain specimens, and over 80% of recently diagnosed GBM patients also demonstrated CMV DNA in peripheral blood samples [74]. Though CMV is known to infect 50-80% of the American population, effective immune control typically limits active disease to the immunosuppressed [75]. It remains unclear if the increased prevalence of active CMV infection in glioma patients plays any role in tumor pathogenesis, or if tumor growth simply provides an environment permissive of local reactivation and propagation of the virus. Regardless, the presence of CMV in these tumors may be important considering it's known potential to modulate growth, invasiveness, and immunological recognition of infected cells [76]. Indeed, active CMV infection has been shown in astrocytes to reduce expression of molecules necessary for antigen presentation, increase the expression of TGF-  $\beta$  and IL-10, and limit the susceptibility of infected cells to apoptotic pathways [77,78]. Elucidation of the impact CMV virus has on the immunosuppressive phenotypes of CNS tumor cells will require further investigation. Nonetheless, the presence of viral antigens only in tumor cells may allow for specific targeting through the use of CMV antigens in CNS tumor vaccines. If in fact active CMV activation contributes to cellular transformation or malignant behavior, then vaccination strategies against its antigens could additionally provide a functionally disabling therapy toward preventing recurrence.

## Immunoediting in CNS Cancer

Analysis of human CNS tumors is largely performed on ex vivo specimens obtained from surgical resection following presentation of clinical deficits. Therefore, the insights gained by these investigations may not be representative of earlier stages in tumor development. Thus, whereas it is possible to study the immunosuppressive environment present in a malignant tumor, the interaction between immune cells and neoplastic tumor precursors throughout tumor development remains obscure. Accordingly, the theory of tumor immunoediting has emerged as paradigm for understanding the dynamics of tumor progression and immunosuppression. Tumor immunoediting proposes three distinct phases: an initial elimination, a period of equilibrium, and finally, cancer cell immune escape [79]. Tumor immunoediting is summarized in Figure 3. Under the

paradigm of immunoediting, developing tumors are believed to contain multiple cancer cell sub-populations with different immunogenic antigen profiles, resulting randomly from genetic instability and rapid proliferation [75]. In the initial elimination phase, cytotoxic immune cells target and eliminate those cancer cells with recognizable antigens and that lack immune evasion mechanisms, leading to the selection of poorly immunogenic and/or immunosuppressive tumor cells. Nevertheless, the elimination of cancer cells is incomplete, and due to either poorly antigenic or immunosuppressive-related gene expression profiles, certain sub-populations of tumor cells are not eradicated. These remaining tumor cells then enter an equilibrium phase, in which exists a dynamic balance between active tumor cell elimination and select tumor cell expansion. Equilibrium is believed to be a long phase in which no clinical manifestations of tumor are perceived. The latent period of equilibrium is interpreted as an editing stage in which host immunity eliminates those tumor cells that is recognizes, while those that are not recognized are therefore selected and survive. Finally, an escape phase occurs when those tumor cells that avoided immune recognition in the elimination and equilibrium phases, either due to novel tumor antigen profiles or immunosuppressive mechanisms, overwhelm the equilibrium state or grow into a symptomatic lesion (Figure 1).



**Figure 3:** Cancer immunoediting paradigm, highlighting the three proposed phases of immunoediting: elimination, equilibrium, and escape. Reprinted with Permission from [74].

Considering the competence of immune surveillance and activation within the CNS, the principles of tumor immunoediting are believed to apply to CNS cancers. Support for the paradigm of immunoediting in CNS cancers comes from few transplant studies, citing the transmission of glioma tumors from liver and kidney organ donors to transplant recipients, and from observations in ongoing immunotherapy trials. The first report of this phenomenon involved a 44 year-old woman with primary biliary cirrhosis who received an orthotopic liver transplant from a 14 year old brain-dead donor with a glial tumor that had infiltrated the pons, pituitary, and spinal cord.



Following 9 months of immunosuppression, the recipient developed several liver lesions that appeared histopathologically similar to that of the donor's glial tumor, suggesting immune escape of glioma cells maintained in quiescent immune equilibrium prior to transplantation [80]. A similar report documented two recipients who each received a kidney from a deceased donor with GBM. Both recipients developed renal masses after approximately 18 months, which upon organ removal were pathologically consistent with GBM [81]. Further evidence comes from current GBM vaccine trials. Analysis of recurrent GBM specimens following use of a vaccine targeting the highly expressed variant EGFRvIII in GBM demonstrated a paucity of EGFRvIII expression, suggesting successful elimination of the EGFRvIII expressing cells, followed by equilibrium and subsequent escape of cancer cells sub-populations which did not express EGFRvIII [82]. Ongoing investigation of the dynamic interactions between immune cells and tumor cells throughout the multi-phasic progression of CNS tumors will test this theory of immunoediting in CNS cancers, and potentially elucidate opportunities to enhance elimination and redirect the eventual failure of equilibrium.

## Conclusion

A comprehensive understanding of the dynamic balance between tumoricidal immunity and tumor-derived immunosuppression which takes place during CNS cancer development is essential for successful immunotherapy of CNS tumors. As this review highlights, many immune players participate in complicated interactions with CNS tumor cells to effect a general suppression of activated tumoricidal immune states. Ever-unfolding insight into the specific mechanisms of these pathological interactions may allow for targeted therapies to augment tumoricidal immune activation and to disable tumor-derived immunosuppressive barriers. Currently, numerous immune-based therapies designed to target many of the pathological mechanisms discussed in this review have demonstrated great promise in preclinical and clinical investigations. The prospect for these immune-based therapies to supplant the invasive and damaging current standards of surgery, radiation, and chemotherapy use to treat many CNS tumors is extremely exciting. Additional insight into shared molecular regulators of immunosuppression, such as STAT-3, the evolution of immune evading antigenic heterogeneity within CNS tumors, and the expression of CMV antigens in transformed cells of CNS cancers may further contribute to new and innovative strategies to combat immunosuppression and stimulate tumoricidal activation states.

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