# Mechanisms of Local Immunoresistance in Glioma

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**KEYWORDS**

• Glioblastoma multiforme • Immunotherapy
• Oncogenic signaling pathway • Immunoresistance

Glioblastoma multiforme (GBM) is a malignant tumor of the central nervous system (CNS) comprising 40% of all primary brain tumors. Of the astrocytomas, GBM is the most malignant, high-grade type of glioma that kills 13,000 Americans every year. Without treatments, most patients face a severe prognosis, surviving fewer than 6 months. The mean duration of survival is only 14 months even after intensive therapy, combining gross total resection, radiation, and chemotherapy.[1,2] Several obstacles prevent standard therapies from effectively fighting malignant gliomas. GBMs exhibit robust proliferation, angiogenesis, genetic instability, and immunosuppression. In addition, it is a very infiltrative tumor that diffuses through white matter tracts and periventricular/perivascular areas, resulting in migration to the contralateral hemisphere.[3] As a result, the tumor cells may migrate far beyond what is visualized radiographically at the time of diagnosis. Thus, although surgical resection can remove the visible tumor mass, it cannot eradicate invasive and migratory cells. These challenges underscore the need for novel strategies to improve the outcome of patients with GBM. Immunotherapy is a strategy that would allow for surveillance and eradication of this local and distant disease.

Another factor that contributes to GBM malignancy is the high degree of genetic instability that generates cellular heterogeneity.[4] This hinders cancer cells from responding equally to radiation and chemotherapy, causing further relapses. In addition, chemotherapy has generally been unsuccessful because of poor drug delivery. The presence of active efflux transporters in the blood-brain barrier (BBB) prevents systemically administrated drugs from entering the brain,[5] thus highlighting the need for new comprehensive strategies to overcome this physical obstacle.[6]

Historically, the CNS has been viewed as immune privileged.[7,8] The CNS was considered unique relative to other organ systems by the virtues of the BBB restricting the migration of immune cells and cytokines into the brain, the absence of a lymphatic drainage system, the presence of a high concentration of immunosuppressive factors, and the lack of major histocompatibility complex (MHC) molecule expression in normal CNS cells.[9–11] However, newer data suggest that the CNS is a perfectly adequate environment for immune responses as evidenced by the presence of both humoral and cell-mediated CNS immunity.[12–14] In addition, lymphocytes have been shown to traffic to normal brain (both naive lymphocytes and activated T cells),[15] by crossing the BBB without antigen specificity.[15–17] Furthermore, many types of lymphocytes appear in the CNS during illness, such as infection or autoimmune processes.[18–20]

## THE TUMOR IMMUNE MICROENVIRONMENT

Many tumors, including GBM, create an immunosuppressive local environment to shield themselves from the body's normal immune response. The immune microenvironment created by GBM

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likely plays a much larger role in immune evasion than the general BBB, which is typically compromised by the tumor. GBM evades the immune system using several strategies: (1) aberrant antigen recognition leading to insufficient immune cell activation, (2) promotion of suppressor immune cells to induce T-cell tolerance or apoptosis, (3) upregulation of co-inhibitory molecules, (4) secretion of immune inhibiting molecules, (5) recruitment of suppressor immune cells, and (6) activation of immunosuppressive pathways.

### *Abnormal Antigen Recognition and Immune Cell Activation*

One mechanism by which GBM evades the immune system is by preventing normal antigen recognition. This process is orchestrated by the MHC, also known in humans as human leukocyte antigen (HLA), which displays fragmented pieces of self or non-self-antigens on the host cell surface. Normally, T cells interact with MHC via the T-cell receptor molecules to determine if the antigen is self or foreign. A second signal is also required for T cells to become fully activated, the costimulatory signal. If this process occurs properly, T cells will ignore self-peptides and react appropriately to the foreign peptides (**Fig. 1**A).

#### *MHC*

Parney and colleagues[21] found that most GBMs expressed low levels of class I MHC and no class II MHC. These data are supported by Lampson's[22] finding that class I MHC can be upregulated in gliomas after interferon $\gamma$ (IFN$\gamma$) exposure in vitro.

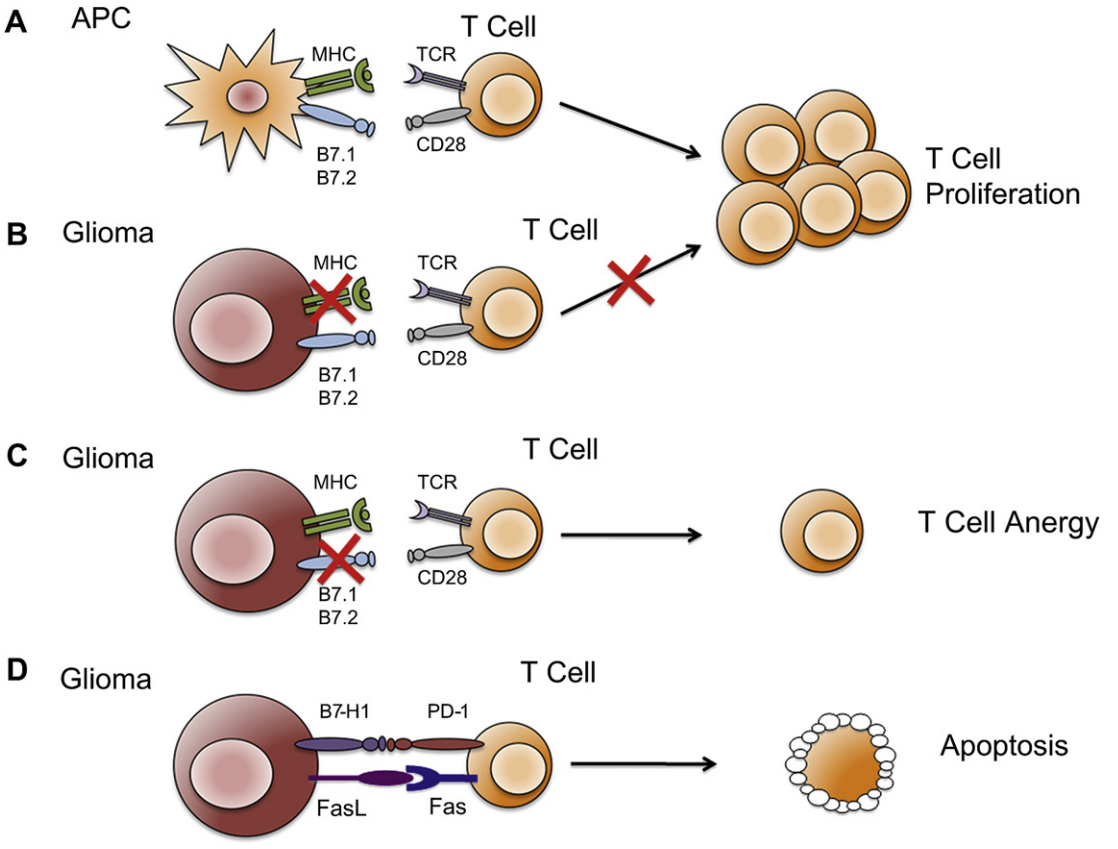


**Fig. 1.** Strategies adopted by glioma cells to inhibit T-cell proliferation and activation by interfering with the antigen presenting process. (*A*) Classical antigen presentation by antigen presenting cells through expression of MHC and costimulatory molecules. T cells interact with MHC via the T-cell receptors to determine if the antigen is self or foreign. When T cells identify non-self-peptides, they start to proliferate and activate to mount an immune response. Tumors disrupt this interaction by (*B*) downregulating the expression of MHC molecules on the cell surface. Loss of MHC molecules blocks the cross talk between tumor cells and the tumor-interacting immune cells. (*C*) Downregulation of the costimulatory molecules, such as B7.1 and B7.2, to induce T-cell anergy. (*D*) Upregulation of inhibitory B7 molecules, such as B7-H1, or death signals, such as FasL, cause T-cell apoptosis via binding with their receptors.

There are several reasons why class I and class II MHC molecules are not expressed on the glioma cell surface. First, gliomas have been reported to express immunoinhibitory factors, such as transforming growth factor β (TGF-β)[23] and prostaglandin $E_2$ ($PGE_2$),[24] that downregulate class II MHC on glioma cells. Second, most GBM lesions express mutated class I HLA molecules. HLA class I antigen loss significantly correlates with tumor grade[25] and with immunotherapy refractory tumors.[26] The antigen processing machinery (APM) components were also investigated, and tapasin expression was found to be downregulated in GBM lesions. Those aberrations seem to be linked with the mutations of HLA class I antigen expression and significantly correlates with the clinical course of the disease. These findings suggest that mutations in HLA class I antigen and in APM components may provide a mechanism for GBM to escape immune recognition and killing by cytotoxic T lymphocytes (CTLs). These findings emphasize the need to monitor HLA class I antigen and APM component expression in GBM lesions when selecting patients for T-cell–based immunotherapy treatment.[25]

### *Costimulatory molecules*

T-cell costimulation is necessary for T-cell proliferation, differentiation, and survival. Activation of T cells without costimulation may lead to T-cell anergy, T-cell deletion, or the development of immune tolerance. CD28, one of the best characterized costimulatory molecules expressed by T cells, interacts with CD80 (B7.1) and CD86 (B7.2) on the membrane of antigen presenting cells (APCs) (see **Fig. 1**A).

In addition to expressing low levels of MHC peptide (see **Fig. 1**B), cancer cells downregulate the costimulatory molecules that are required for activating a proper immune response (see **Fig. 1**C). Lack of T-cell costimulation is another mechanism by GBM to avoid immune surveillance. So far, B7 molecule expression is found to be absent on glioma cells.[27] In addition, peripheral blood T cells from patients with glioma typically show a high degree of anergy to GBM antigens that results from the absence of costimulatory molecules.

Studies have also shown that the receptors for the costimulatory molecules on tumor-infiltrating APCs are downregulated. Researchers have shown that the human glioma-infiltrated microglia or macrophages (GIMs) completely lack CD80/CD40 expression and show minimal CD86 expression, which could explain their inability to properly activate naive T cells.[28] Another study conducted on intracranial RG2 glioma-bearing rodents showed that GIMs from brain tumors respond differently to general activators, such as CpG oligodeoxynucleotides (CpG ODN) and IFNγ/lipopolysaccharide (LPS), when compared with those from normal brain. CpG ODN induced the upregulation of B7 molecules but had little effect on MHC-II expression, whereas IFNγ/LPS had the opposite effect. Both upregulations were significantly lower in tumor-associated GIMs, in comparison with GIMs from normal brain. Further studies are necessary to understand if these diminished effects are a result of the local GBM immunocompromising environment, abnormal signaling, or mutated receptor expression on the tumor-infiltrating GIMs.[29]

The B7 costimulatory family includes activating and inhibiting molecules that regulate immune responses positively and negatively. Among the latter group, B7-H1, one of the newly identified B7 family member, has been shown to provide negative signals that control and suppress T-cell responses.[30] The regulation of B7-H1 seems to be pivotal in shaping the immune response to tumors because it can exert costimulatory and immune regulatory functions.[31] Although B7-H1 has been shown to mediate tumor evasion by binding to programmed death-1 (PD-1) receptor, additional counter receptors can also control the functions of B7-H1.[32]

Human and rodent cancer cells and immune cells in the cancer microenvironment have been shown to upregulate expression of inhibitory B7 molecules. Analysis of multiple glioma cell lines and human specimens have also shown high levels of B7-H1 (see **Fig. 1**D).[27,33] This high level of expression reduces glioma cell immunogenicity by suppressing T-cell cytokine production and activation. A study by Parsa and colleagues[34] demonstrates a potential relationship between B7-H1 and the phosphatase and tensin homolog-phosphatidylinositol 3-kinase (PTEN-PI3K) pathway. They show that the loss of PTEN and the activation of the PI3K-pathway lead to elevated post-transcriptional expression of B7-H1. This represents a novel mechanism of immunoresistance mediated by B7-H1, further demonstrating the importance of this molecule in tumor evasion of immune surveillance. B7-H1 has even been reported to correlate with the malignancy grade of gliomas.[35] These studies demonstrate the potential benefits of using neutralizing antibodies specific for B7-H1 and PD-1 in the treatment of patients with malignant brain tumors.

## *Deregulation of Cell-mediated Immunity*

During the past 3 decades, many studies of patients harboring glioma revealed that these

individuals exhibit a broad suppression of cell-mediated immunity in a manner similar to those involved in autoimmunity processes. Studies have suggested that the immune cells from patients with GBM behave in a manner that is reminiscent to autoimmune diseases, such as cutaneous anergy to common bacterial antigens,[36] lymphopenia,[37] impaired antibody production,[37] and abnormal delayed-type hypersensitivity response to common recall antigens or neoantigens in vivo.[37,38] It seems that the lymphocytes in patients with GBM present intrinsic cellular abnormalities that render potentially reactive T cells unresponsive. Peripheral blood lymphocytes (PBLs) obtained from patients with GBM did not proliferate or minimally proliferated in response to mitogen stimulation in vitro. Elliott and colleagues[39] showed that PBLs obtained from patients with GBM have approximately 6 times fewer phytohemagglutinin (PHA)-reactive lymphocytes than those obtained from normal subjects. These lymphocytes fail to expand into a pool of proliferating cells in vitro. In addition, the supernatant fluids of PHA-stimulated lymphocytes obtained from patients contain a substantial reduction of interleukin-2 (IL-2) and IFN$\gamma$ compared with lymphocytes obtained from normal donors. Moreover, T cells obtained from patients with GBM are unable to offer helper activity in allogeneic pokeweed mitogen cultures in vitro.[39–41] This comprehensive depression in cellular immune function is not typical of head trauma or other tumors of the brain. Hence, it must be the complex GBM tumor microenvironment that compromises T-cell compartments and their functions.

In addition to the alterations of the intrinsic activation pathways in T cells, GBM also induces accumulation of immunosuppressive cells in the microenvironment. GBM promotes impaired immunocompetence by taking advantage of the normal immunosuppressive mechanisms by stimulating the proliferation of the regulatory T ($T_{reg}$) cells. In vivo depletion of $T_{reg}$ cells causes severe autoimmune disease, which can be reversed by reconstitution.[42] Moreover, the regression of tolerogenic tumors after depletion of $T_{reg}$ cells has been observed in vivo.[43]

Fecci and colleagues[44] reported an unbalanced ratio between $CD4^+$ T cells and $T_{reg}$ cells in GBM. Although both fractions were greatly reduced in patients with malignant glioma, $T_{reg}$ cells often represented most of the CD4 population. It is well known that $T_{reg}$ cells can inhibit T-cell activation and proliferation by downregulating IL-2 and IFN$\gamma$ production in the target cells.[25,28,29,45–48] This would also explain the shift from $T_H1$ to $T_H2$ cytokines, which propagate the regulatory phenotype.[49,50] As a demonstration of this, depletion of $T_{reg}$ cells in vitro reestablishes the normal CD4 functions in the T cells that are isolated from patients with GBM and reverses the cytokine production to the $T_H1$ type.[44] Tumor tolerance induced by $T_{reg}$ cells is also common in other solid tumors.

In addition to $T_{reg}$ cells, there are other suppressive cell types in the tumor microenvironment. Recruitment of suppressive myeloid cells, such as regulatory dendritic cells (DCs), characterized by indoleamine-pyrrole 2,3 dioxygenase (IDO) expression[51] and myeloid-derived suppressor cells (MDSCs) at the tumor site is another way to inhibit immune responses.[52]

Munn and colleagues documented IDO expression in human and murine myeloid DCs. $IDO^+$ DCs catabolize tryptophan to block local T- lymphocyte clonal expansion, causing T-cell death by apoptosis, anergy, or immune deviation.[51,53–56] Suppressive myeloid cells would directly contribute to induction of $T_{reg}$ cells in the tumor microenvironment and vice versa; $T_{reg}$ cells can induce IDO expression in DCs and effectively convert them into regulatory DCs.[57]

MDSCs also infiltrate tumors, inhibiting immune response and facilitating tumor growth and metastasis.[58] MDSCs inhibit T cell activation by anti-CD3 and superantigen, and by secretion of reactive nitrogen compounds (peroxynitrites) and immunosuppressive cytokines (TGF-$\beta$).[52] Human MDSCs were originally described in patients with head and neck cancer[59] and in the peripheral blood of patients with renal cell carcinoma.[60] Tumor-infiltrated MDSCs have been described in mouse GL261[61] and rat T9 glioma models.[62] In the latter example, the authors reported that MDSCs were recruited at the brain T9 tumor site after subcutaneous vaccination with irradiated T9 glioma cells, which inhibited T-cell function and resulted in tumor progression.

In addition to GBM-induced mechanisms, immunosuppression can also be iatrogenic. Corticosteroids may cause immunosuppression by inhibiting cytokine production and causing sequestration of $CD4^+$ T cells.[63] Newer data suggest, however, that such immunosuppression may be dose dependent; and at therapeutic levels, corticosteroids may not interfere with immunotherapy.[64] Chemotherapy can also contribute to the inhibition of the immune response to tumor. Temozolomide, for example, can cause $CD4^+$ lymphopenia,[65] which may negatively affect immunotherapeutic approaches that use a $CD4^+$ T-cell response. Newer agents, such as rapamycin, inhibit the T-cell proliferative cytokine IL-2.[63]

### *Immunosuppressive Factors*

The tumor microenvironment modulates various cytokines and chemokines expressed by tumor cells or lymphocytes. The glioma microenvironment contains very high levels of immunosuppressive cytokines, which contribute to impaired lymphocyte response in patients with glioma (**Fig. 2**).[66]

#### *TGF-β*

One of the most well-characterized immunosuppressive factors is TGF-β, originally called glioblastoma cell-derived T-cell suppressor factor (see **Fig. 2**).[67] TGF-β regulates inflammation, angiogenesis, and proliferation.[68] Glioblastoma is known to produce high levels of TGF-β in the microenvironment, where it inhibits T-cell and B-cell proliferation, activation,[69,70] and maturation and function of professional APCs.[71] In particular, TGF-β directly inhibits CTL function by blocking the production of cytotoxic molecules, IFNγ, and Fas ligand (FasL).[72,73] This may also explain the inactive state of the tumor-infiltrated T cells. Furthermore, TGF-β is responsible for the downregulation of MHC class II on glioma cells,[23] which serves as another mechanism of tumor escape. TGF-β is an important growth factor for glioma cells expressing TGF-β surface receptor,[74] and it also seems to play a role in maintaining the $T_{reg}$ cell phenotype.

#### *IL-10*

The role of IL-10, known as cytokine synthesis inhibitory factor, is similar to TGF-β.[75] IL-10 is secreted by glioma cells[76] and $T_{reg}$ cells[77]; it

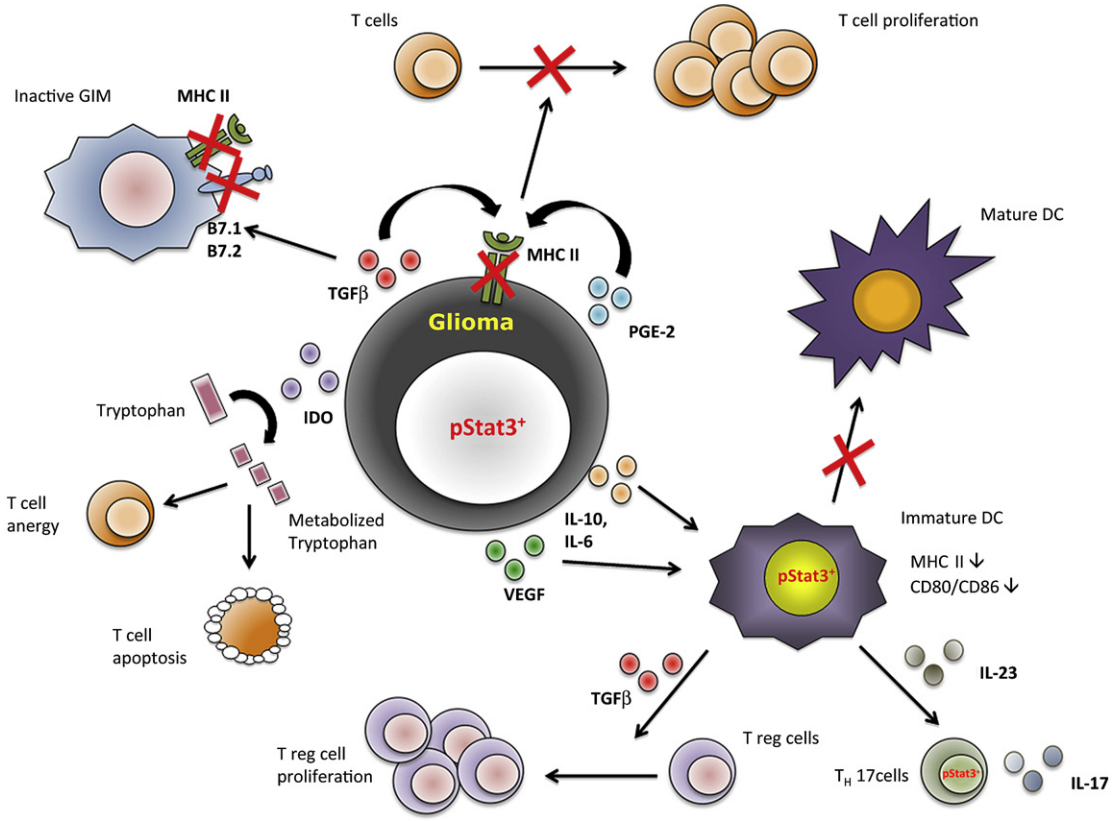


**Fig. 2.** Immunosuppressive factors secreted by glioma cells in the tumor microenvironment. (Starting from the top and proceeding clockwise) TGF-β and $PGE_2$ downregulate class II MHC molecules on glioma cells and on GIMs. As a consequence, the antigen presenting process is compromised and T cells cannot proliferate. IL-10, IL-6, and VEGF are STAT3-dependent cytokines that represent potent STAT3 activators. The tumor microenvironment has many immunosuppressive activities, and these activities are responsible for activation of STAT3 in immune cells. When DC progenitors become $STAT3^+$, they remain immature and are unable to express class II MHC and costimulatory molecules. Therefore, they cannot efficiently prime T cells, and they in turn start to secrete more inhibitory factors, including TGF-β and IL-23, which promote $T_{reg}$ cells and $T_H17$ cells accumulation, respectively. $IDO^+$ gliomas are able to actively metabolize tryptophan, and lack of tryptophan in the microenvironment blocks T-cell proliferation. In this condition, T cells can become anergic or undergo apoptosis.

inhibits production of IFN$\gamma$ by lymphocytes and tumor necrosis factor $\alpha$ by monocytes and downregulates class II MHC on GIMs.[76] IL-10 also induces glioma cell proliferation and motility in vitro.[78] Controversially, IL-10 has been shown to inhibit brain tumor growth in vivo.[79] New studies are necessary to clarify the conflicting roles of this cytokine in the tumor microenvironment.

Another important immunosuppressive quality of IL-10 is the ability to induce signal transducer and activator of transcription 3 (STAT3) in DC progenitors and in other immune cells, including innate immune cells and T cells (see **Fig. 2**).[80] Immunostimulatory molecule expression is reduced in immune cells that show constitutive activation of STAT3. Also, their ability to mount an antitumor immune response is defective. Moreover, they seem to produce immunosuppressive factors, such as TGF-$\beta$, IL-23, and more IL-10, that contribute to the accumulation of $T_{reg}$ cells and possibly $T_H17$ cells in the tumor microenvironment.

### *$PGE_2$*

$PGE_2$ has profound effects on glioma and immune cells (see **Fig. 2**). It promotes tumor cell invasion, angiogenesis, and motility. It also downregulates MHC class II.[24] On the other hand, $PGE_2$ downregulates $T_H1$ cytokine production and upregulates $T_H2$, enhancing $T_{reg}$ phenotype and proliferation.[81] It also suppresses T-cell activation, proliferation, and the antitumor activity of lymphokine-activated killer cells.[82]

### *Vascular endothelial growth factor*

Angiogenesis is responsible for the growth of small localized neoplasms into larger growing and potentially metastatic tumors. Although GBM rarely metastasizes, it almost always recurs locally because of diffuse infiltration resulting from angiogenesis.[83] Angiogenesis is regulated by vascular endothelial growth factor (VEGF) binding to its specific receptors, flt-1 (fms-related tyrosine kinase 1) and flk-1 (fetal liver kinase 1). These receptors are only expressed on tumor endothelial cells, and their interaction with VEGF induces proliferation and migration in situ.[84] Numerous studies have demonstrated the prevalence of VEGF expression and its isoforms in GBM.[85] In vitro and in vivo studies have confirmed a correlation between tumor grade and VEGF expression in gliomas. In addition, studies in animal models have shown that inhibiting VEGF function inhibits growth of glioma cells and causes regression of blood vessels.[83] Within GBM, cells adjacent to necrotic areas are thought to upregulate VEGF, secondary to hypoxia. VEGF expression has been shown to strongly correlate with hypoxia.[86]

In a similar way to IL-10, VEGF has been well characterized as an inhibitor of DC maturation and activation (see **Fig. 2**).[87]

### *IDO*

IDO is a kynurenine pathway enzyme that catalyzes the catabolism of tryptophan,[53] an amino acid essential for T-cell proliferation and differentiation. It has been shown to play various roles within the immune system.[51,88] Uyttenhove and colleagues[89] detected positive expression of IDO in various human tumor specimens, including GBM (see **Fig. 2**). They demonstrated that $IDO^+$ tumors were successful at evading the immune response. However, they were also able to reverse these effects by using an IDO inhibitor, clearly indicating that this protein may play a possible role in tumor evasion of the immune system. Miyazaki and colleagues[90] demonstrated the same IDO mechanism at work in 4 human GBM cell lines. They demonstrated that an IDO inhibitor, 1-methyltryptophan (1MT), effectively prevented the depletion of tryptophan. In addition, combining 1MT with chemotherapeutic drugs augmented the inhibitory effect of these agents on cell growth and tryptophan degradation. These studies indicate that IDO could potentially be a useful target for immunotherapy against GBM by preserving tryptophan levels for T cells.

## *Activation of Immunosuppressive Pathways*

Several oncogenic signaling pathways are constitutively upregulated in GBM. They contribute to tumor progression, resistance to therapies, and tumor immune evasion.

### *STAT3*

GBM presents several signal transduction pathways that are overly activated, such as phosphoinositide-3 kinase, Akt, Ras, mitogen-activated protein kinases, and receptor tyrosine kinases, including epidermal growth factor receptor and VEGF receptor.[65,91] All of these pathways actively stimulate the promotion and the progression of glioma cells. It is known that they converge to specific transcription factors, including STAT3. Aberrant activation of STAT3 has been found in many cancer types,[80,92–94] including GBM.[95] STAT3 activation in tumors prevents apoptosis and promotes cellular proliferation, angiogenesis, and tissue invasion.[80] In glioma cells, STAT3 triggers the expression of antiapoptotic factors, such as Bcl-2, Bcl-XL, Mcl-1, survivin, and cFlip.[96,97] Knockdown of STAT3 expression by

siRNA causes apoptosis in several glioma cell lines but not in primary human astrocytes.[97]

In addition to promoting oncogenesis, STAT3 plays an important role in immune evasion by inhibiting the expression of $T_H1$ mediators[98] and stimulating production of diverse immunosuppressive factors,[73] such as IL-10 and VEGF. This inhibits the induction of a tumor-specific T-cell response[99] and also retains DCs in their immature state, turning them into tolerogenic DCs[100] that are able to promote expansion of $T_{reg}$ cells.[101]

STAT3 has been found to be constitutively activated in tumor-infiltrating DCs and myeloid cells,[73] most probably due to the presence of IL-10 and VEGF, which are potent STAT3 activators in the tumor microenvironment (see **Fig. 2**).[98] STAT3 activity in DCs inhibits the expression of MHC class II molecules, B7-1, B7-2, and IL-12 secretion,[98] thus preventing their maturation and affecting their ability to activate tumor-specific T cells and natural killer (NK) cells. Hussain and colleagues[102] described a similar tolerogenic phenotype on GBM infiltrated microglia or macrophages and their inability to properly activate T cells. Blocking STAT3 with a small molecule inhibitor can reverse immune tolerance in patients with GBM. In particular, costimulatory molecules can be upregulated on GIMs and the production of IL-2, IL-4, IL-12, and IL-15 can be increased, thus inducing proliferation of T cells.[103]

New tumor-infiltrated T-cell populations have been described, and they all have the capacity of secreting IL-17.[104] IL-17 T cells have been originally described in the pathogenesis of autoimmune disease.[105,106] STAT3-induced IL-6/TGF-β costimulation is necessary to promote IL-17 differentiation, and IL-23 is necessary to maintain the IL-17 phenotype.[107–109] Because tumor-infiltrated myeloid cells are the principal source of IL-23[110] and IL-23 is responsible for tumor-associated inflammation and angiogenesis,[108] it is reasonable to speculate that IL-17 T cells might have a potential role in cancer development.

### *Fas/FasL*

Fas is a member of the tumor necrosis factor receptor family.[111] It is an apoptotic receptor that binds to FasL. This binding triggers an intracellular cascade resulting in cell death.[112,113] However, a study involving GBM shows that Fas receptor activation results in cell survival and proliferation rather than apoptosis.[114]

FasL expression has been detected in various tumor types.[115–118] Although FasL expression has been predominantly identified in activated immune cells, such as T cells, phagocytes, and NK cells,[112,113,119,120] its role in immune reaction suppression still remains unclear.

Cancer cell acquisition of FasL expression has been shown to deliver death signals to activated Fas-positive T lymphocytes.[121–124] This counterattack hypothesis is thought to grant the tumor an immune-privileged status. This concept originated from initial studies in transplantation, which demonstrated that the Fas-FasL interaction was fundamental to maintaining an immune-privileged status.[125,126] However, later studies contradicted these previous results, showing that FasL expression resulted in rapid rejection accompanied by inflammation.[127–130] Other contradictory studies report that FasL can also have proinflammatory and antitumoral effects.[131,132] These conflicting findings regarding Fas-FasL highlight that this system is not fully understood and that certain environmental conditions, tumor type, activated pathways, and presence or absence of immune cell populations are involved in this response.[133,134]

In GBM, the use of conventional chemotherapeutic drugs (such as camptothecin and etoposide) can sensitize the tumor cells to Fas-dependent apoptosis.[135,136] The use of decoy receptor 3, a soluble decoy for FasL, has also been shown to reduce the number of tumor-infiltrating CD4 and CD8 T cells in a 9L gliosarcoma model.[137] The administration of topotecan, a Fas-enhancing chemotherapeutic agent, before immunotherapy may also amplify apoptotic receptors, further sensitizing glioma cells for immune clearance.[138] These results demonstrate the potential benefits of combination therapy involving chemotherapy with immunotherapy in patients with glioma by focusing on Fas-FasL.

### *Galectin-1*

Galectin-1, a prototype member of the galectin family, is a homodimeric adhesion molecule and carbohydrate-binding protein with affinity for β-galactosides.[139] Galectin-1 plays a multifaceted role in promoting brain tumor malignancy.[140] This protein contributes to the invasive and migratory potential,[141–144] angiogenesis,[145] and chemoresistance[146] of glioma cells. Galectin-1 expression levels in glioma have even been shown to directly correlate with tumor grade.[142,147]

Galectin-1 also plays an important role in regulating immune cell homeostasis and inflammation.[148–150] Galectin-1 promotes apoptosis of activated T cells,[151–154] induces partial T-cell activation,[155] and blocks proinflammatory cytokine secretion.[156,157] Galectin-1 also contributes to tumor-induced immunosuppression in vitro and in vivo.[147] In patients with head and neck squamous cell carcinoma, Le and colleagues[158]

demonstrate an inverse relationship between galectin-1 expression and the presence of T cells, suggesting that galectin-1 is a negative regulator of T-cell activation and survival. These results support the concept that galectin-1 contributes to immune privilege of tumors by negatively regulating the survival of effector T cells. Galectin-1 is also thought to play similar roles in gliomas, yet its immunosuppressive role has not been determined in these particular tumors.

## SUMMARY

Gliomas are specialized in evading the host immune system and current immunotherapies. An important component of this efficient immune escape is harbored in the complexity of the glioma microenvironment. Many immunosuppressive mechanisms are active simultaneously, and they can self-sustain themselves by creating a positive feedback loop and enhancing their effects. The glioma-derived suppressor factors generate a shift from $T_H1$ to $T_H2$ cytokines, resulting in deregulation of cell-mediated immunity, accompanied by accumulation of immunosuppressive type of cells. In addition, regulatory cell secretion of active immunosuppressive factors and activation of immunosuppressive pathways allow for tumor escape mechanisms at multiple levels. These phenomena provide difficult challenges in designing immunotherapies. However, it is clear that targeting multiple points in the immunosuppressive response and continual improvements in current vaccines will lead to improved immunotherapies. In addition, one has begun to appreciate that pathways and mechanisms affect multiple processes. For example, STAT3 affects cell proliferation and angiogenesis in addition to immunotherapy. Continuous studies of the glioma cells and their cross talk with the immune cells are paramount to overcome tumor tolerance and in the development of strategies to cure hopeless patients with GBM.

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