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Combating immunosuppression in glioma

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Abstract

Despite maximal therapy, malignant gliomas have a very poor prognosis. Patients with glioma express significant immune defects, including CD4 lymphopenia, increased fractions of regulatory T cells in peripheral blood and shifts in cytokine profiles from Th1 to Th2. Recent studies have focused on ways to combat immunosuppression in patients with glioma as well as in animal models for glioma. We concentrate on two specific ways to combat immunosuppression: inhibition of TGF-β signaling and modulation of regulatory T cells. TGF-β signaling can be interrupted by antisense oligonucleotide technology, TGF-β receptor I kinase inhibitors, soluble TGF-β receptors and antibodies against TGF-β. Regulatory T cells have been targeted with antibodies against T-cell markers, such as CD25, CTLA-4 and GITR. In addition, vaccination against Foxp3 has been explored. The results of these studies have been encouraging; combating immunosuppression may be one key to improving prognosis in malignant glioma.

Keywords

CD25; CTLA-4; cytotoxic T-lymphocyte antigen 4; Foxp3; GITR; glioblastoma multiforme; glioma; glucocorticoid-induced tumor necrosis factor receptor-related protein; immunosuppression; regulatory T cells; TGF-β; transforming growth factor-β

Glioma epidemiology & prognosis

Glioblastoma multiforme (GBM) is the most common type of malignant brain tumor and has an extremely poor prognosis. Median survival for GBM remains less than 15 months, despite maximal surgery, radiotherapy and chemotherapy [1]. Various methods of immunotherapy, such as dendritic cell vaccines and adoptive T-cell therapy, have recently

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been explored [2] in an attempt to harness the host's immune system to target tumor cells. With few exceptions, results have been disappointing. There is a dire need for effective therapies against brain tumors, and combating the systemic immunosuppression that is seen in patients with GBM as a means of improving anti-tumor immunity may be one of the forthcoming strategies in neuro-oncology.

Immune privilege in the brain

Two phenomena that may contribute to the failure of standard therapies and pose potential obstacles to immunotherapy in malignant glioma are the blood–brain barrier (BBB) and the concept of 'immune privilege' in the brain. Despite the long-standing belief that the BBB prevents passage of molecules and cells into the brain parenchyma [3], immune cells [4] and antibodies [5] have been shown to infiltrate into the CNS, indicating that the BBB is not an absolute barrier. Trafficking of immune cells and transport of immune-related molecules do occur, although in a highly controlled manner [6].

The original concept of the BBB helped shape the belief that the CNS is an immune-privileged system that is unable to mount an immune response against foreign pathogens or diseased tissue. The BBB is no longer felt to be solely responsible for this immune privilege; there are several other important factors related to immune surveillance that contribute to this phenomenon. For example, microglia residing in uninflamed brain parenchyma can act as antigen-presenting cells (APCs), but are much less effective than macrophages in the periphery [7]. The presence of other APCs in the CNS parenchyma is unclear. Major histocompatibility complex (MHC) class I and II expression is also reduced in the CNS [8], reducing the likelihood that an antigen will be presented to a T cell. These features, along with the lack of a traditional lymphatic drainage system in the CNS, lead to muted activities in the afferent arm of the adaptive immune response characterized by compromised or diminished activation of T cells relative to other organs [9]. These conditions allow for the control of inflammation in the CNS, which is essential, in a tissue with little capacity to repair itself following injury. However, the immune-privileged environment of the CNS and the tightly regulated BBB also contribute to a setting where immune surveillance of the CNS is poor, and glioma cells can grow relatively unchallenged by the immune system. Unopposed tumor growth is further exacerbated by the microenvironment created by the tumor itself, which enables systemic suppression of immune responses [10].

Glioma immunobiology

It has been known for many years that patients with malignant glioma exhibit a variety of immune defects, many of which are related to impaired T-cell function. These patients demonstrate a severe and systemic immunosuppression (for review, see [11]), as evidenced by impaired cell-mediated immunity [12–14], downregulation of interleukin-2 (IL-2) production and interleukin-2 receptor (IL-2R) signal transduction [15–17], cytokine dysregulation that favors Th2 responses and decreased capacity to induce a delayed-type hypersensitivity reaction [18,19]. $CD4^+$ T-cell lymphopenia is common in patients with malignant glioma; the remaining $CD4^+$ T-cell population is prone to anergy and contains an increased percentage of regulatory T cells (T_{regs}) [10]. In addition, peripheral blood lymphocytes show a reduced ability to proliferate in response to T-cell mitogens, anti-CD3 antibody stimulation and T-cell-dependent B-cell mitogens [16,20–22]. Furthermore, the glioma cells themselves secrete immunosuppressive factors, such as IL-10 [23] and transforming growth factor-β (TGF-β) [11]. The result of these numerous and combined immune defects is a state of severely curtailed anti-tumor immunity in which the host has difficulty mounting an effective immune response against the tumor.

There is some evidence suggesting potential endogenous immunity in patients with glioma. For example, a history of allergies has been found to negatively correlate with risk of glioma [24]. It is postulated that the immune response of individuals who are prone to allergies is largely humoral, and thus able to mount a response against potential tumor antigens and prevent glioma from developing [25]. Increased thymic $CD8^+$ T-cell production has been shown to be positively correlated with age-associated prognosis in patients with glioma. Thymic $CD8^+$ T cells are capable of recognizing common tumor antigens expressed by glioma, and may play a significant role in the tumor-specific immune response [26]. Other studies have found that after dendritic cell vaccination, improved prognosis correlates with the absence of bulky, progressing tumor, low levels of TGF-β2 [27], and increased $CD8^+$ T-cell response.

Combating immunosuppression

Immune privilege in the CNS, the BBB and systemic immunosuppression in patients with glioma all create obstacles to successful therapy by creating an environment of poor anti-tumor immunity. Immunotherapy faces additional problems, such as paucity of tumor-specific antigens, overcoming self-tolerance and tumor heterogeneity. Combating immunosuppression in these patients is one way of potentially improving efficacy of all modes of therapy. There are numerous, interesting ways to approach the problem of immunosuppression in patients with glioma, such as targeting cyclooxygenase-2 [28], STAT3 [29] or B7-H1 protein [30]. We will focus on two important strategies that have been investigated recently in glioma and other types of cancer and have shown promise: inhibition of TGF-β signaling and modulation of T_{regs}.

TGF-β

TGF-β and its receptors are expressed in almost every cell in the body; its isoforms play wide-spread roles in cellular homeostasis, proliferation and differentiation, wound healing and extra-cellular matrix production [31]. TGF-β plays a complex role in malignant glioma. In the early stages of tumorigenesis, TGF-β may act as a tumor suppressor by inhibiting tumor growth [32]. TGF-β has been shown to inhibit proliferation [33] and DNA synthesis in normal astrocytes [34] as well as to antagonize the mitogenic function of several growth factors [35]. However, glioma cells eventually lose their ability to respond to the growth-inhibition signals of the TGF-β ligand [34]. TGF-β, which is secreted by microglia present within the tumor [36], may then act to enhance tumorigenicity via several less direct mechanisms. For example, the cytokine has been implicated in increasing glioma motility by upregulation of integrin molecules [37], and in enhancing angiogenesis by stimulating expression of growth factors, such as vascular endothelial growth factor (VEGF) [38]. Perhaps most importantly, TGF-β induces a T_{reg} phenotype in peripheral naive T cells [39,40], which may be the mechanism by which TGF-β modulates immunosuppression in glioma and other tumors. Thus, inhibition of TGF-β may be a means of breaking tolerance or enabling an antitumor immune response, as well as a mechanism to prevent further glioma motility or invasiveness.

TGF-β signaling begins with active TGF-β binding to the TGF-β receptor II (TGF-βRII). TGF-βRII then recruits and activates TGF-β receptor I (TGF-βRI), which leads to activation of TGF-βRI kinase and subsequent phosphorylation of the proteins Smad2 and Smad3. Smad2 and Smad3 form a complex with Smad4, which enters the nucleus to regulate gene expression. Membrane-bound TGF-β receptor III (TGF-βRIII) is capable of presenting TGF-β to the TGF-βRII, but is not essential to the TGF-β signaling process [41]. There are a number of strategies for inhibiting TGF-β, including translation inhibition by antisense oligonucleotide technologies, prevention of downstream signaling by interfering with TGF-

β receptor kinase activities and direct targeting of the growth factor with antibodies or soluble receptors. These methods and others have been studied to determine their effectiveness at reducing TGF-β-induced immunosuppression in glioma animal models and in clinical trials, as discussed below.

Antisense oligonucleotides

One way of inhibiting TGF-β is the use of anti-sense oligodeoxynucleotide (AS-ODN) technology. AS-ODNs are synthetic sequences of nucleic acid that are complementary to the mRNA of a particular gene product. Upon administration, the AS-ODN will prevent the translation of targeted mRNA and the subsequent production of its gene product (here, TGF-β). In a preclinical study using 9L glioma in Fischer 944 rats, the combination of intracranial TGF-β-2 AS-ODN administration and vaccination with irradiated glioma cells not only inhibited TGF-β protein production *in vivo* as expected, but also significantly prolonged survival times when compared with either vaccine alone or no therapy [42].

AS-ODN technology has moved into clinical Phase I/II trials with a TGF-β-2 antisense compound called AP 12009 in patients with malignant glioma. AP 12009 therapy resulted in significantly decreased TGF-β-2 secretion from patient-derived primary glioma cell culture, reduced tumor cell proliferation in a dose-dependent manner and increased cytotoxicity of patient peripheral blood mononuclear cells (PBMCs) directed against autologous tumor in *in vitro* assays. Some patients experienced prolonged survival or delayed progression of disease when compared with historical controls [43]. AP 12009 has the obvious potential to be a significant contributor to the therapy regimen of patients with malignant glioma through its action of inhibiting TGF-β-induced immunosuppression.

TGF-β receptor I kinase inhibitors

TGF-β signal inhibition is a strategy that has recently been employed with the use of compounds that block enzymatic reactions in the TGF-β signaling pathway. SD-208, a TGF-βRI kinase inhibitor, reversed some of the TGF-β-induced immune defects associated with glioma. Administration of SD-208 following tumor inoculation in a syngeneic murine model of glioma (SMA-560 in VM/Dk mice) increased median survival, and also increased tumor infiltration by natural killer (NK) cells, $CD8^+$ T cells and macrophages [44]. SX-007, another TGF-βRI kinase inhibitor, was effective in inhibiting TGF-β signaling, as measured by inhibition of the phosphorylation of Smad 2/3. SX-007 also improved median survival in VM/Dk mice previously inoculated with SMA-560 [45]. The success of these preclinical trials will hopefully lead to clinical trials in patients with glioma sometime in the near future.

Soluble TGF-β receptors

Soluble forms of TGF-β receptors, such as TGF-βRII and TGF-βRIII, also called betaglycan, may also modulate the cytokine's effects. Ordinarily these receptors are transmembrane molecules, but extracellular domains can break off and bind TGF-β, preventing it from binding to transmembrane receptors and initiating signal cascades [46]. Recombinant forms of these extra-cellular domains have been used to 'sop up' extracellular TGF-β. It has been shown that expression of TGF-βRIII is decreased in human breast cancer, and that the decrease is associated with breast cancer progression. Stable transfection of mammary cancer cells with TGF-βRIII increased TGF-βRIII expression and resulted in delayed and decreased metastases, decreased angiogenesis and decreased invasiveness of breast cancer cells in animal models [47]. TGF-βRIII has also shown to be reduced in ovarian cancer [48] and prostate cancer [49], although the state of TGF-βRIII in glioma is

not yet known. If it is downregulated in glioma, administration of betaglycan may prove to be a successful therapy. Clearly, more studies are needed.

Targeting TGF-β receptors

While the administration of soluble receptors has not yet been examined in glioma, studies have examined the effects of downregulating TGF-βRII in glioma cells. Wesolowska *et al.* designed plasmid-transcribed small hairpin RNAs (shRNAs) that downregulated TGF-βRII expression and inhibited the subsequent signaling and transcriptional pathways in transiently transfected human glioblastoma cells. In addition, when these cells were placed in nude mice, tumorigenicity of the cells was significantly reduced [36]. This study did not examine the effects of shRNA on immune function, but such data would be of great interest. These techniques should be advanced into glioma murine models and, if successful, into clinical trials.

Anti-TGF-β antibodies

Anti-TGF-β antibodies bind to active extracellular TGF-β and prevent ensuing intracellular signaling through the TFG-β receptor. After years of preclinical study [42,50,51], the use of anti-TGF-β antibodies in cancer therapy has reached Phase I clinical trials [101] for patients with renal cell carcinoma and metastatic melanoma. Neutralizing antibodies to TGF-β have been shown to impede immunosuppression in animal models [52], and thus, this may be a useful approach for patients with glioma as well. Our laboratory is currently conducting pre-clinical studies using anti-TGF-β antibody therapy in a syngeneic glioma murine model.

One of the most important systemic effects of TGF-β is the induction of T_{regs}. It is highly probable that the decrease in immunosuppression seen with successful blockade of the action of TGF-β is at least in part, mediated by reduction in the number or function of T_{regs}. Another way to accomplish this effect is, of course, to target T_{regs} directly.

Regulatory T cells

T_{regs} are important in maintaining self tolerance and in the prevention of autoimmunity by inhibition of T-cell activation and proliferation [53]. Characteristic of T_{regs} in both mice and humans is the high expression of surface markers CD25 (IL-2R-α-chain), constitutive expression of cyto-toxic T-lymphocyte antigen 4 (CTLA-4), over-expression of glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), and the expression of the transcriptional regulator Foxp3 [54,55]. T_{regs} have been shown to downregulate IL-2 [56] and interferon-γ (IFN-γ) production [57], decrease Th1 cytokine production and increase Th2 cytokine production in target cells [10,58]. The net effect of these T_{reg} activities is a switch from cell-mediated to humoral immunity, and an unfavorable environment for anti-tumor immunity.

As discussed above, patients with glioma have profound defects in their $CD4^+$ T-cell compartment, of which T_{regs} play an important role. Despite overall $CD4^+$ T-cell lymphopenia, increased T_{reg} fractions are seen in the peripheral blood of patients with malignant glioma [10], as well as in patients with other types of cancers [59,60]. This increase in T_{regs} may correlate to increasing cancer stage [61]. The increased fraction of T_{regs} in peripheral blood in patients with glioma is necessary and sufficient to induce the T-cell defects associated with T_{reg} activities, including decreased T-cell responsiveness and Th1 to Th2 cytokine shifts [10].

Abrogating the action of T_{regs} may be essential to successful treatment of glioma. Several studies have examined different methods of inhibiting T_{reg} function in glioma, as well as other tumor types. Many of the strategies employed to reduce T_{reg} function target CD25,

which makes up one subunit (α) of the IL-2R that is present on the surface of T_{regs} and activated T cells.

Targeting CD25

Anti-CD25 Antibody—Anti-CD25 monoclonal antibody (mAb) has been used in several studies as a means of inhibiting T-cell function in glioma models. In the syngeneic murine Vm/Dk model for SMA-560, systemic administration of anti-CD25 mAb inhibited the suppressive action of T_{regs}, although without completely eliminating the T_{reg} fraction. Inhibition of T_{reg} function led to increased lymphocyte proliferative responses and IFN-γ production, as well as specific lysis of glioma cell targets *in vitro* [62]. Anti-CD25 mAb prolonged survival concurrent with tumor rejection when administered prior to tumor implantation, and even resulted in long-term survival for some animals when administered after the tumor had become established [10]. When T_{reg} inhibition was combined with tumor RNA-pulsed dendritic cell vaccination, 100% of mice rejected their tumors [62]. Similar phenomena were witnessed when the peripheral blood of patients with malignant glioma were combined with anti-CD25 mAb *in vitro*. This eliminated the proliferative defect, decreased Th2 cytokine production and normalized Th1 cytokine production [10].

Another study examined anti-CD25 mAb in a GL261 glioma model in C57BL/6 mice. Administration of anti-CD25 mAb significantly reduced the number of T_{regs} found within the tumor and led to delay or complete prevention of tumor development in some mice [63].

Clinical trials using the humanized anti-CD25 mAb daclizumab to inhibit T_{regs} in patients with GBM are nearing completion in our laboratory with promising results. Treatment has resulted in a decrease in circulating T_{regs} without impacting CD4 and CD8 counts. The impact on immune and clinical responses is still being assessed (John H Sampson, Duke University School of Medicine, NC, USA. Unpublished Data).

LMB-2—Another potential drug that can be used to target T_{regs} is the CD25-specific immunotoxin, LMB-2, which is formed by fusion of the single-chain Fv fragment of the anti-CD25 mAb with a recombinant form of the *Pseudomonas* exotoxin. LMB-2 was combined with peptide vaccination to treat patients with melanoma, and showed a significant but transient reduction in the number of T_{regs}, both in peripheral blood and within the tumor. However, no patient experienced regression of tumor, and combination of LMB-2 with peptide vaccine produced no additional specific immune response compared with peptide vaccine alone [64]. Like anti-CD25 mAb, LMB-2 has the potential for eliminating or inactivating non-T_{reg} $CD25^+$ T cells, although this is less of a concern given the shorter half-life (approximately 4 h versus approximately 20 days) of LMB-2. As LMB-2 contains an immunotoxin, it may also cause both a direct cytotoxic effect and a delayed immunologic response [65]. Despite the lack of clinical response, this compound appears promising, and preclinical studies examining the drug in an animal model for glioma would be interesting.

ONTAK—Several other investigators have examined denileukin diftitox (ONTAK: Ligand Pharmaceuticals, San Diego, CA, USA), a fusion protein of IL-2 and diphtheria toxin that binds to the IL-2R (made up of CD25, CD122 and CD132) in various types of cancers. The drug has been shown in some studies to deplete T_{regs} in patients with T-cell lymphoma [66] and in patients with melanoma [67], but other studies have shown no clinical or immunological effect [68]. Again, no studies have been done using this drug in a glioma model, and given the conflicting results in other types of cancer, it is unclear whether this therapy holds promise for GBM.

Targeting other T_{reg} markers

Anti-CTLA-4 blockade—Another possible approach to reduce immunosuppression in glioma is via CTLA-4 blockade. CTLA-4, a homologue of CD28 and a member of the immunoglobulin superfamily, is a transmembrane protein that binds to ligands B7–1 and B7–2 that are expressed on APCs [69]. CTLA-4 is constitutively expressed on T_{regs}, and is also expressed by activated T cells. Through interactions with co-stimulatory molecules on other cells, CTLA-4 acts to decrease T-cell responsiveness. Anti-CTLA-4 antibodies have been studied as potential therapeutics for glioma. Dosing SMA-560 ghoma-bearing Vm/DK mice with anti-CTLA-4 antibody led to long-term survival in a high percentage of the treatment group, as well as to a reversal of the CD4 T-cell defects (e.g., normalized $CD4^+$ T-cell and T_{reg} levels). Surprisingly, systemic CTLA-4 blockade had no direct effect on $CD4^+CD25^+$ T_{regs}, but rather demonstrated all of its effect on $CD4^+CD25^-$ effector T cells [70]. It is hypothesized that CTLA-4 works as a co-stimulatory molecule to the $CD4^+CD25^-$ T cells and induces their proliferation, allowing them to overcome T_{reg} suppression.

Humanized CTLA-4 blocking antibodies are now available and strategies targeting CTLA-4 have been employed with some success in melanoma clinical trials in conjunction with peptide vaccines [71,72] or IL-2 treatment [73]. We are currently evaluating CTLA-4 blockade alone and in combination with tumor-specific vaccines in patients with malignant glioma.

Anti-GITR agonism—GITR, a member of the tumor necrosis factor receptor (TNFR) superfamily, is a co-stimulatory molecule that is expressed in several immune cells, including T cells, NK cells and APCs. GITR is activated by GITR ligand (GITRL), which is found on APCs. GITR is over-expressed on T_{regs}, but is also present on $CD4^+CD25^-$ T cells. It acts to increase T-cell receptor (TCR)-induced T-cell proliferation and cytokine production, which in turn abrogates T_{reg} suppression of $CD4^+CD25^-$ T cells. In T_{regs}, GITR activation can have variable effects, either inhibiting T_{reg} activity or increasing T_{reg} proliferation and suppression [74]. An agonistic anti-GITR antibody has been shown to be capable of inducing tumor-specific immunity in tumor-bearing mice and of eradicating established fibro-sarcoma [75]. The mechanisms of action are not yet completely understood, but it is possible that, like CTLA-4, anti-GITR antibody exerts its main effects by activating effector T cells so they are able to resist suppression by T_{regs}. Our laboratory is currently examining anti-GITR antibody in a syngeneic glioma murine model with impressive preliminary results. No humanized anti-GITR antibody has been developed, however, and some evidence exists that signaling through GITR may have different effects in mice and humans [76].

Vaccination against Foxp3—Finally, in a unique twist on immune modulation, a recent report employing vaccination against Foxp3 has shown promising results in an animal model [77]. Foxp3 is an intracellular transcription factor that regulates suppression and is expressed by both $CD4^+$ and $CD8^+$ T_{reg} subsets. In this study, mRNA-transfected dendritic cells were expressed and presented a truncated function-inactivated Foxp3, whose forkhead structure had been disrupted and one of two nuclear localization signals deleted. Vaccination of C57BL/6 mice with these cells resulted in a Foxp3-specific cytotoxic T-lymphocyte (CTL) response, although there was no increase in survival. Immune effects of vaccination were comparable to administration of anti-CD25 antibody, but this approach may be more specific to T_{regs}, as CD25 is also expressed on some activated T cells.

Combination approaches—It is likely that the most successful therapies will combine several modalities. Already, it has been shown that administration of a combination of anti-CTLA-4 antibody and anti-GITR antibody has synergistic effects on glioma tumor rejection

than either therapy alone [75]. Likewise, anti-CTLA-4 antibody and anti-CD25 antibody also worked synergistically when administered concurrently in a glioma model [63].

Conclusion

Despite continuing research in various modalities of therapy, the prognosis of malignant glioma remains poor even with maximal therapy. Combating immunosuppression in patients with glioma may increase host anti-tumor immunity and lead to increased efficacy of concomitant therapies and, hopefully, a better prognosis. Thus far, preclinical and clinical studies targeting TGF-β and T_{regs} (via CD25, CTLA-4, GITR and Foxp3) have shown varying degrees of success. Methods to reduce production or signaling of TGF-β have been the most varied in nature, as well as the most abundant in glioma models. Certainly, more specific studies need to be conducted in order to elucidate methods for reducing immunosuppression in glioma via anti-CD25 blockade, anti-CTLA blockade and anti-GITR agonism. In addition, therapeutic compounds, such as betaglycan, LMB-2 and ONTAK have not yet been examined in glioma and may prove to be beneficial.

Methods to reverse immunosuppression in glioma must be considered in the framework of the current standard of care, which currently includes pre- and post-operative steroids and chemotherapeutic agents, both of which are known to affect cell-mediated immunity. Administration of anti-immunosuppressive therapy must be carefully timed so as to optimize the immune response. There is preliminary evidence of successful combination therapy of immunotherapy and temozolomide, [78] despite the obvious concern that chemotherapy-induced lymphopenia would exclude immunotherapy as an option. It may be possible to exploit the lymphopenia that follows temozolomide treatment by using immunotherapy to inhibit T_{regs} during that period. Anti-TGF-β or anti-T_{reg} therapy may fit nicely into this paradigm by enhancing the depletion or inactivation of T_{regs}.

Future perspective

Targeting TGF-β and T_{regs} are certainly not the only methods by which we can combat immunosuppression in glioma. For example, restoration of the natural balance of Th1 and Th2 cytokines may occur by any number of methods, and this may improve anti-tumor immunity. Administration of IL-2, IL-7 or IFN-γ or blocking of IL-10 or prostaglandin E2 (PGE2) should be investigated as potential means of combating immunosuppression in glioma. IL-7 administration was shown to increase T-cell numbers and decrease T_{reg} fraction in humans, *in vivo* [61]; this has potential therapeutic implications for patients with lymphopenia, such as those with glioma. In addition, we may be able to develop therapies to exploit endogenous immunity in patients with glioma as we learn more about this phenomenon. For example, it may be valuable to explore ways to increase thymic production of T cells or to enhance the humoral immune response associated with allergies.

In the past several years, many experiments have attempted to use immunotherapy to enhance the immune response in patients with glioma. The preliminary success of the anti-immunosuppression studies described above may spur a shift in focus from enhancing immune response alone to a combined approach that includes the reversal of immune suppression. Some studies have already shown that immunotherapy combined with immunosuppressive techniques is more successful than immunotherapy alone [29,62,79]. It remains to be seen how successful reducing immunosuppression will be when combined with traditional therapies, but so far, a future as an adjunct to immunotherapy is promising. Patients with GBM will likely require a comprehensive treatment regimen that uses multiple approaches to fight tumor; reversing immunosuppression may be one of the important keys to eventually improving prognosis for these patients.

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Website

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Executive summary

Immunosuppression in glioma

- Patients with malignant glioma show multiple immune defects, including, but not limited to, CD4 lymphopenia, increased fraction of regulatory T cells (T_{regs}) in peripheral blood, decrease in Th1 cytokines and increase in Th2 cytokines, and a switch from cell-mediated to humoral immunity.
- These changes help to create an environment of poor anti-tumor immunity and likely contribute to the poor prognosis of the disease.
- Recent studies have focused on ways to combat this immunosuppression, for example, by inhibiting TGF-β signaling and by modulation of T_{regs}.

Inhibition of TGF-β signaling

- Antisense oligodeoxynucleotide (AS-ODN) technology involves the administration of nucleotide sequences that complement and bind to TGF-β mRNA, preventing its translation into gene product. TGF-β-2 AS-ODN prolonged survival in a rat glioma model. In Phase I/II clinical trials, the TGF-β-2 AS-ODN compound AP 12009 was found to be well-tolerated and safe when administered to patients with recurrent glioma; prolonged survival was seen in several patients.
- TGF-β receptor I kinase inhibitors have been used to prevent the downstream signaling of TGF-β after the growth factor binds to its transmembrane receptors. The drug SD-208 showed reversal of TGF-β-induced immune defects associated with glioma and increased median survival in a syngeneic tumor model for glioma. A similar drug, SX-007, inhibited the phosphorylation of Smad 2/3 and improved median survival in the same murine model.
- Soluble TGF-β receptors are extracellular regions of TGF-β receptor II and III that break off and bind to extracellular TGF-β, preventing binding to a transmembrane receptor and subsequent intracellular signaling. Betaglycan (soluble TGF-βRIII) has been shown to reduce metastasis in breast cancer in animal models, but has not yet been evaluated in glioma.
- Anti-TGF-β antibodies work in a manner similar to soluble TGF-β receptors, by binding to extracellular TGF-β and preventing binding to a transmembrane receptor. Phase I clinical trials studying anti-TGF-β antibodies have been initiated in renal cell carcinoma and melanoma. Preclinical trials examining anti-TGF-β antibodies in glioma are currently underway.

Modulation of regulatory T cells

- Anti-CD25 antibody administration in a syngeneic glioma murine model inhibited the suppressive action of T_{regs}, increased lymphocyte proliferative responses and IFN-γ production and prolonged survival.
- LMB-2 is a CD25-specific immunotoxin that, when combined with peptide vaccination, significantly reduced T_{reg} numbers in peripheral blood and within the tumors of patients with melanoma. No tumor regression was seen and this drug has not been examined in glioma.
- Denileukin difitox (ONTAK) is a fusion protein between fragments of diphtheria toxin and recombinant IL-2 that binds to IL-2 receptors. The drug has

inconsistently reduced T_{reg} numbers in patients with T-cell lymphoma and melanoma, but has not been evaluated in glioma.

- Anti-CTLA-4 blockade in a syngeneic glioma murine model resulted in long-term survival and reversal of the CD4 T-cell defects. Humanized CTLA-4 blocking antibodies have shown some success in treating patients with melanoma; trials evaluating anti-CTLA-4 blockade in patients with malignant glioma are underway.
- Vaccination against Foxp3 was examined in a mouse glioma model and showed a Foxp3-specific CTL response, but no increase in survival.

Conclusion

- Combating immunosuppression through TGF-β inhibition and T_{reg} modulation in glioma may be one key to increasing the efficacy of both traditional therapies and immunotherapy, and potentially leading to improvement in patient prognosis.