Published in final edited form as:

Neurosurg Clin N Am. 2010 January ; 21(1): 125–137. doi:10.1016/j.nec.2009.08.012.

The Role of Tregs in Glioma-Mediated Immunosuppression: Potential Target for Intervention

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Abstract

The role of regulatory T cells (Tregs) in mediating immune suppression of anti-tumor immune responses is increasingly becoming appreciated in patients with malignancies - especially within the malignant glioma patient population. This review will discuss the role and prognostic significance of Tregs within glioma patients and will delineate potential approaches for their inhibition that can be used either alone or in combination with other immune therapeutics in clinical trials and in the clinical settings of recurrent and/or residual disease.

Keywords

regulatory T cells; glioblastoma multiforme; central nervous system; immunotherapy; vaccine; prognosis

Overview of immunosuppressive Tregs

The function of the immune system is to recognize foreign materials in the body and distinguish them from normal body tissues and cells. Immune responses consist of cell-mediated (T cells, natural killer cells, and phagocytes) and/or humoral (B cells, antibodies, and complement) responses modified and regulated by cytokines. Antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages take up antigens, partially degrade them, and present them to T cells in the context of major histocompatibility complex (MHC) molecules. To fully activate the adaptive immune response, the T cells must receive two signals: one through the T cell receptor and the other through the co-stimulatory receptor CD28, which recognizes the co-stimulatory molecules CD80 and CD86 expressed on the surface of APCs. Failure to do so

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will result in T cell anergy [1]. T cells have a number of functions, including potentiating cytotoxic T cell responses (CD4⁺ helper T cells [Th1 cells]), assisting B cells in the production of antibodies (CD4⁺ helper T cells [Th2 cells]), recognizing and destroying virally infected or tumor cells (CD8⁺ effector T cells), and determining the level of reactivity in the immune system (CD4⁺/CD25⁺/[forkhead box P3⁺] Foxp3⁺ regulatory T cells [Tregs]).

Tregs are a physiologic subset of CD4⁺ T cells that curtail the function of T cells, B cells [2, 3], DCs [4-6], monocytes and/or macrophages [6], natural killer T cells [7], and natural killer cells [8,9]. Tregs potently inhibit T cell cytokine secretion and proliferation by down regulating interleukin (IL)-2 and interferon (IFN)- γ production [10-14], increase Th2 cytokine skewing [15], directly curtail the generation and expansion of endogenous and/or induced immune responses by suppressing proinflammatory cytokine production [16-24], and apparently play a significant role in hindering immunity to tumor-associated antigens [25,26]. Furthermore, studies of murine models of immunogenic tumors have shown that adoptively transferred Tregs inhibit tumor-reactive effector T cells and that elimination of Tregs *in vivo* enhances antitumor immunity [15]. More specifically, in murine tumor challenge models of established gliomas, *in vivo* depletion of Tregs has resulted in enhanced tumor rejection and increased median survival durations [27-29].

Since the discovery of Tregs [30], our understanding of the types and functions of these cells has increased greatly. Tregs are classified into three subtypes based on their induction site, cytokine profile, and respective cell surface markers: natural Tregs (nTregs), Th3 cells, and Tr1 cells (Fig. 1) [31-34]. nTregs (CD4⁺/CD25⁺/Foxp3⁺) are thymically derived, bind with intermediate affinity to the MHC/peptide complex, and are capable of recognizing both self-generated and foreign antigens. Upon their exportation to the periphery from the thymus, nTregs exert their effect on peripheral effector T cells primarily via cell-to-cell contact. Although the mechanism by which nTregs exert their effects on the effector T cells has yet to be fully elucidated [31-34], it likely results from downregulation of IL-2 cytokine production and may involve membrane-bound cytotoxic T lymphocyte antigen-4 (CTLA-4), a negative regulator of T cell activation, a member of the CD28/B7 immunoglobulin superfamily that is constitutively expressed on Tregs [35], and whose expression is up-regulated in activated T cells [36,37]. . The development of nTregs is regulated by the Foxp3 gene in CD4⁺/CD25⁺ T cells [38]. The primary role of nTregs is suspected to be maintenance of a constant homeostatic balance by curtailing the effects of autoreactive T cells in noninflammatory settings [32].

Tregs in circulating peripheral blood include not only nTregs differentiated in the thymus but also Foxp3⁺ Tregs generated extrathymically by the conversion of naïve T cells via chronic encounters with antigens present in suboptimal doses [39] or by the suppressive cytokine milieu secreted by the glioma. *In vitro* experiments have demonstrated that peripheral T cells retain the ability to induce Foxp3 expression upon T cell receptor cross-linking in the presence of TGF- β [40] or by the CD28/B7 interaction [41]. However, the overall contribution of the peripheral conversion of Tregs to immune suppression and its functional significance are not clear. Investigators have described two populations of peripherally induced CD4⁺ Tregs: Tr1 and Th3 cells. Tr1 cells require IL-10 for induction, predominantly secrete IL-10, and to a lesser degree TGF- β and INF- γ . Tr1 cells inhibit naïve, memory, and helper T cells and the ability of DCs to induce T cell proliferation [42]. In contrast, Th3 cells [43] are induced by IL-10 and TGF- β [44] and predominantly secrete TGF- β and IL-10 at levels lower than Tr1 cells do. It is suspected that these induced Tregs (iTregs) play a primary role in mitigating pathological immune responses such as those seen in cases of infection and autoimmune-mediated inflammation [32].

Biological role of Tregs in patients with glioma

Studies have implicated Tregs mediate immunosuppression in patients with a number of different malignancies, including ovarian, pancreatic, breast, colorectal, lung, and esophageal cancer [25,45,46]. For example, Curiel *et al.* [25] showed that the Treg fraction was higher in ascites of patients with ovarian cancer compared to that of patients with nonmalignant ascites. The authors also showed that Tregs preferentially migrated to the tumor microenvironment induced by CCL22 secreted by the tumor cells and macrophages. Furthermore, Tregs inhibited the function of tumor-infiltrating T cells by inhibiting production of IL-12 and INF- γ . These findings were instrumental in implicating a role for Tregs in tumor-mediated immunosuppression.

Patients with malignant gliomas have severe defects in host humoral and cellular immune responses [47]. These defects are characterized by dramatic reductions in both CD4⁺ T cell number [27] and function [48,49] and a disproportionate presence of immunosuppressive Tregs [27]. This increase in Treg fractions corresponds with a decrease in effector T cell functions. Furthermore, the removal of the Treg fraction from T cells obtained from patients with glioblastoma multiforme (GBM) restores T cell proliferation and cytokine responses to normal levels [27]. Moreover, *in vitro* depletion of Tregs from peripheral blood results in the successful reversal of effector T cell function, including increased T cell proliferation and a switch from Th2 to a Th1 (IL-2⁺/tumor necrosis factor- α (TNF- α)/INF- γ) cytokine profile. These findings demonstrate the important role of Tregs in glioma-mediated immunosuppression.

In the glioma microenvironment, the anti-tumor effector T cells can be critically suppressed and/or overwhelmed by Tregs. Researchers have obtained human glioma tissue during surgery, dissociated the tumors into single-cell suspensions, and stained them for the CD8⁺ and CD4⁺ subsets of T cells [50]. They found that tumor-infiltrating CD8⁺ T cells were phenotypically CD8⁺ and CD25⁻, indicating that these effector cells were not activated or proliferating. The CD4⁺ T cells were more numerous than CD8⁺ T cells within the gliomas, and the majority of CD4⁺ T cells were Tregs as evidenced by positive intracellular staining for Foxp3. In another study, the CD4⁺/CD25⁺/Foxp3⁺ T cells were found only in gliomas, whereas Tregs were absent from control brain tissue specimens [51]. The presence of Tregs within the glioma microenvironment is secondary to the elaboration of the chemokine CCL2 by gliomas which induce the migration of Tregs [52]. Finally, in murine models of syngeneic murine glioma, investigators have observed a time-dependent accumulation of Foxp3⁺ Tregs in brain tumors [53]. These data indicate that Tregs can not only inhibit the initial systemic anti-tumor immune activation but also prevent effector T cell responses in the tumor microenvironment and thus are a potential therapeutic target for inhibition.

Prognostic significance of Tregs in patients with glioma

T cells in the central nervous system (CNS) of healthy humans are a rare finding. However, during inflammatory responses, T cells are evident within the CNS. T cells require activation prior to entry into the CNS [54], but antigen specificity is not necessary for this entry. T cell infiltrates are commonly identified in human gliomas [55], and multiple studies have attempted to correlate the intensity of T cell infiltration with survival [55-57]; however, this prognostic significance has not been seen consistently [58]. Of note is that these types of immunohistochemical assays used in the aforementioned studies do not take into account the functional activity of these T cells or the influence of the immune inhibitory Tregs. Thus, although these T cells are activated in the systemic circulation, their functional activity likely becomes impaired upon entry into the glioma microenvironment [59]; thus, the fact that the T cells presence in a glioma is not a definitive prognostic marker is not surprising.

Researchers showed that CD8⁺ T cells were present in the majority of glioma specimens regardless of tumor grade; however, the number of patients with CD4⁺ T cell populations (including Tregs) increased as the tumor grade increased (39% for World Health Organization [WHO] grade II tumors to 73% for WHO grade III tumors to 98% for grade WHO grade IV tumors) [60]. Foxp3⁺ Tregs are not usually seen in normal brain tissue specimens and are very rare in patients with oligodendroglioma (WHO grade II), mixed oligoastrocytoma (WHO grade II), or anaplastic oligodendroglioma (WHO grade III) (Fig. 2). In contrast, 39% of the anaplastic mixed oligoastrocytoma specimens (WHO grade III), 53% of the anaplastic astrocytoma specimens (WHO grade III), 48% of the GBM specimens (WHO grade IV), and 83% of the gliosarcoma specimens (WHO grade IV) had Foxp3⁺ Tregs [60]. Thus, Tregs were more common in high-grade astrocytic gliomas than in low-grade oligodendroglioma-type tumors.

Because the presence of Foxp3⁺ Tregs correlates with the overall malignant behavior of astrocytic tumors, the expectation that the presence of Tregs in the tumor microenvironment will act as a negative prognostic indicator is reasonable. Univariate analysis demonstrated that, similar to other established parameters, such as the Karnofsky performance score, patient age, and tumor grade, the presence or absence of Foxp3⁺ Tregs and absolute number of Foxp3⁺ Tregs per tumor sample were prognostic factors. However, a multivariate analysis performed to account for confounding factors, such as patient age and Karnofsky performance score, found that the presence of Foxp3 Tregs did not have a prognostic impact [60]. Although some cancers may mediate immunosuppression predominantly via Tregs, high-grade gliomas have multiple mechanisms of mediating immunosuppression [47]. Thus, the lack of a prognostic impact of one mechanism in this setting, such as the presence or absence of Foxp3⁺ Tregs, is not entirely surprising and emphasizes the redundancy of immunosuppressive pathways. Furthermore, this type of study does not account for the prognostic influence of Tregs in the systemic, peripheral blood compartment.

Mechanisms of modulating Treg responses

It is widely recognized that tumors evade the host immune response through a number of mechanisms, including elaboration of immunosuppressive cytokines, alteration of signal transduction [61], and the induction of Tregs. Thus, at the core of the development of more effective immunotherapeutic strategies for brain tumors is simultaneous stimulation of more potent immune responses against these tumors while overcoming immunosuppressive mechanisms induced by the tumors themselves. Overcoming Treg-induced immunosuppression can be achieved using a variety of approaches, including administration of denileukin diftiox (Ontak; a recombinant protein of diphtheria toxin and IL-2) [62], cyclophosphamide (CTX) [63], an anti-CD25 antibody (targeting the IL-2 receptor) [29], CTLA-4 blockade (inhibits co-stimulation) [28], and signal transduction and activator of transcription (STAT)-3--blocking agents that also block transcriptional activation of Foxp3 [64,65]; inhibition of intratumoral Treg trafficking (i.e., inhibition of CCL2) with temozolomide [66]; and, nonspecifically, lymphodepletion to augment immunological responses, which investigators have described in both murine models [63,67] and human patients with cancer [68,69]. Antitumor responses enhanced by lymphodepletion may be secondary to the removal of competition at the surface of APCs [70], enhanced availability of cytokines that augment T cell activity (such as IL-7 and IL-15) [71], and/or the depletion of immune inhibitory Tregs [58].

Developing an optimal approach to modulating or suppressing the Treg population for therapeutic purposes in cancer patients is an area of controversy. CTX, an alkylating agent with therapeutic effects against tumors at high doses, preferentially inhibits Tregs at lower doses. CTX can abolish the function of CD4⁺/CD25⁺/Foxp3⁺ T cells and enhance cytotoxic T cell responses [72]. Treatment with CTX prior to antitumor vaccination results in activation of

tumor-specific CD8⁺ T cells [73]. When CTX is administered at sub-tumoricidal doses in combination with IL-12 in mice, it improves immune response and eradicates large established sarcomas [74]. This combination enhances CD4⁺ T cells, CD8⁺ T cells, and macrophage infiltration in tumors and skews the immune responses to the Th1 phenotype [74]. Other investigators have reported similar findings of treatment of established melanomas with IL-2, IL-1, and CTX in mice [68]. In addition to its induction of immunity to new antigens, CTX can overcome immune tolerance. For example, administration of CTX in mice bearing established plasmacytomas resulted in a cure in 92% of the mice, and further studies demonstrated that the cured mice experienced rejection of a subsequent tumor challenge [75, 76]. The mechanisms of efficacy appeared to include both generation of CD8⁺ cytotoxic T cells and up-regulation of expression of B7-1 (a co-stimulatory molecule).

Multiple clinical trials have demonstrated enhanced immune responses and improved clinical efficacy when CTX was administered prior to immunotherapy [77,78]. For example, when CTX was administered to augment an autologous melanoma vaccine in patients with metastatic melanoma, there were enhanced delayed-type hypersensitivity responses [79]. Researchers have seen similar potentiation of immune responses in metastatic breast cancer and renal cell carcinoma cases. CTX has also been used in a phase I clinical trial of glioma patients that demonstrated evidence of clinical efficacy [80]. In this trial, Plautz and colleagues gave a single dose of CTX to patients with newly diagnosed glioma prior to administration of adoptively transferred T cells harvested from the patients' lymph nodes. They conducted this study without the benefit of our current understanding of the influence of CTX on Tregs; thus, the contribution of CTX to the clinical efficacy of this immune therapeutic strategy is unknown. Despite the use of CTX in clinical trials, the timing and dosing of CTX for optimal Treg inhibition [81, 82] relative to each type of vaccination or immunotherapy strategy still needs further refinement.

Researchers have also studied temozolomide, which is capable of suppressing Tregs, in clinical trials of immunotherapy for GBM. Temozolomide is the standard of care for GBM and an alkylating agent that causes cell death by inducing cell-cycle arrest at G2/M phase and, likely, autophagy without apoptosis [83]. In addition to inhibiting the proliferation of lymphocytes, temozolomide can deplete Tregs [66,84] and inhibit trafficking of Tregs into the glioma microenvironment [52]. The use of temozolomide has proven to be beneficial in combination with a peptide vaccine targeting epidermal growth factor receptor variant III in a phase II clinical trial in patients with newly diagnosed GBM [85]. Another clinical trial, sponsored by Northwest Biotherapeutics (Bethesda, MD), is using GBM patient autologous DCs for an immunotherapy in combination with temozolomide in a similar manner. The use of temozolomide as a possible Treg modulator has particular appeal in the glioma patient population since it is the current standard of care.

Inactivation of Tregs by treatment with an anti-CD25 antibody [29,53,86] or CTLA-4 blockade [46,53] have demonstrated efficacy in murine glioma models. Various studies support the notion that CTLA-4 blockade can enhance antitumor immune responses by limiting suppression of effector T cell responses [87] either by directly activating effector T cells or indirectly inactivating Tregs. Although some studies have shown that CTLA-4 blockade fails to suppress Tregs [87], others have indicated that Tregs and CTLA-4 blockade act independently and that the effects of CTLA-4 blockade are not focused on Tregs [35,88,89], such that CTLA-4 blockade may be synergistic with strategies that inhibit Tregs [89]. In one study of systemic delivery of CTLA-4 blocking monoclonal antibodies in mice with well-established malignant astrocytomas recapitulating the biology of human gliomas [90], the treatment produced a long-term survival rate of 80% without induction of autoimmune encephalomyelitis [28]. CTLA-4 blockade also re-established normal CD4⁺ T cell counts and abrogated increases in the Treg fractions elicited by the tumors. Significant increases in total

and CD4⁺ T cell counts were also observed in individual mice treated with anti-CTLA-4 when compared to mean counts in the untreated group, suggesting a mechanism independent of tumor destruction. Furthermore, CTLA-4 blockade restored CD4⁺ T cell proliferative capacity and enhanced antitumor immune responses. Interestingly, the benefits of CTLA-4 blockade appear to be bestowed exclusively upon activated CD4⁺/CD25⁻ T cells but not the Tregs. In the study described above, the CD4⁺/CD25⁻ T cells obtained from treated mice demonstrated both improved proliferative responses and Treg resistance, whereas Tregs obtained from the same mice remained anergic *in vitro* and exhibited no restriction of their suppressive effect on effector T cells not treated with CTLA-4 monoclonal antibodies. This absence of a direct effect of anti-CTLA-4 antibodies on Tregs strengthens the notion that CTLA-4 blockade may be synergistic with strategies designed to remove Tregs [89]. Although eliminating suppression of endogenous antitumor immune responses through the removal of Tregs may enhance tumor immune clearance, it is accompanied by a potential risk of inducing autoimmunity, although investigators did not find this in murine models. Strategies that induce Th17 responses but not necessarily Treg inhibition likely are the primary mechanisms of inducing CNS autoimmunity [91]. Nevertheless, CTLA-4 blockade has demonstrated safety and significant efficacy as an antitumor strategy in a variety of animal models [92-95].

The greatest overall clinical experience of Treg modulation in cancer patients is with antibodies that abrogate the function of CTLA-4, including some clinical studies that have included patients with metastatic brain tumors [96,97]. These fully human antibodies (produced by Pfizer [New York, NY] and Bristol-Meyers Squibb [New York, NY]/Medarex [Princeton, NJ]) were created using strains of mice with engineered human immune systems. Their use in clinical trials, mostly for melanoma and prostate cancer [98,99], has been associated with a spectrum of autoimmune-associated side effects, such as dermatitis, enterocolitis, hepatitis, uveitis, and hypophysitis [100], which, in some cases, are associated with clinical response and sustainable progression-free survival [97,101]. Specifically, in one study in which an anti-CTLA-4 antibody was administered with gp100 melanoma-associated antigens to patients with melanoma, 36% of patients with at least grade 3 autoimmune toxic effects had a clinical response, whereas only 5% of those with no autoimmune toxic effects had a clinical response (overall response was 13% regardless of autoimmune toxicity) [102]. This CTLA-4 blockade has been shown to enhance both tumor-specific humoral and cytotoxic responses in patients [103]. Another study found that anti-CTLA-4 immunotherapy with IL-2 in a phase I/II clinical trial in patients with melanoma had an objective response rate of 22% [104]. Interestingly, anti-CTLA-4 therapy has generated clinically meaningful antitumor immunity without autoimmune toxic effects in patients with melanoma or ovarian cancer who were previously vaccinated with irradiated autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor (GVAX) [105], suggesting that anti-CTLA-4 therapy may be beneficial in previously vaccinated patients. Unfortunately, anti-CTLA-4 therapy has yet to be studied in patients with malignant glioma, but given the aforementioned data, it may be appropriate for use in those glioma patients who have previously received vaccination or immune therapeutics who may be in early stages of progression to provide an immunological “boost.” Furthermore, we propose that the therapeutic efficacy of epidermal growth factor receptor variant III-targeted vaccination [106,107] may be enhanced in combination with CTLA-4 blockade in patients with GBM who have residual disease through reversal of Treg-mediated immunosuppression and may enhance vaccine-induced and endogenous antitumor immune responses.

In the case of anti-CD25, two different immunotoxins have been created that could be exploited for inhibiting Tregs; one is linked to ricin A (RFT5.SMPT-DGA) [108,109], whereas the other is linked to *Pseudomonas* exotoxin (LMB-2) [110]. Investigators have administered these two immunotoxins in patients with heavily pretreated refractory Hodgkin disease and hairy cell leukemia, respectively, and observed clinical responses. The toxic effects of RFT5.SMPT-DGA included weakness, edema, dyspnea, and myalgia [111], whereas those of LMB-2

consisted primarily of transaminase-level elevations and fever [112]. As with the anti-CTLA-4 approaches, neither of these agents or the other anti-CD25 preclinical approaches that have been investigated in murine models [29,53,86] have been attempted in glioma patients but these agents could also be used in combination with other immune therapeutics or in the setting of residual disease.

In a study of patients with metastatic renal carcinoma, treatment with Ontak (anti-IL-2) specifically depleted Tregs without inducing toxic effects and significantly improved stimulation of tumor-specific T cell responses when those patients were stimulated with tumor RNA-transfected DCs [113]. Without tumor vaccination, researchers have shown that Ontak is efficacious as a single agent against relapsed/refractory B-cell non-Hodgkin lymphoma and cutaneous T cell lymphoma [114]. However, Ontak has not been particularly effective in depleting Tregs in patients with melanoma [61], a tumor of similar neuroectodermal derivation as glioma. Furthermore, Ontak targets IL-2, which is expressed in effector T cells; because the effector T cell immune population is already compromised in patients with GBM [115-117], further suppression of the antitumor effector T cell population may be deleterious in these patients.

Authors have reported that targeting of the Toll-like receptor (TLR) elicits effective antitumor responses against various neoplasms, including experimental brain tumors [118], which may partially neutralize the effects of Tregs. For instance, one study has shown that the stimulation with synthetic or natural ligands for human TLR8, and the subsequent transfer of TLR-8 ligand-stimulated Tregs into tumor-bearing mice led to the enhancement of anti-tumor immunity [119]. Furthermore, a study showed that TLR stimulation by CpG-oligodeoxynucleotides in murine GL261 gliomas enhanced CD8⁺ T cell mediated immune responses and demonstrated a marked increase in the ratio of CD4⁺ effector T cells CD4⁺/Foxp3⁺ Tregs [120]. However, TLR agonists may not be able to reverse the other mechanisms of immunosuppression in patients with glioma, including immune inhibitory microglia/macrophages present in the tumor microenvironment [50].

Future directions

An emerging approach to modulating Tregs is administration of agents that block or disrupt Notch signaling and STAT-3 inhibitors. Notch plays a role in regulating the responses of T cells and can induce the differentiation of CD4⁺ T cells into Tregs [121]. However, which member(s) of the Notch family that must be blocked is unclear. As described previously [64, 65], the STAT-3 inhibitors are a new class of compounds that are potent inhibitors of Tregs that will soon be tested in clinical trials. Researchers have shown that STAT-3 activation is required for both TGF- β and IL-10 production by CD4⁺ T cells [122]; both of these factors are necessary for the generation of tumor-associated Tregs. In addition, a study has shown that IL-2 regulates Foxp3 expression in human CD4⁺/CD25⁺ Tregs by via STAT-3 binding of the first intron of the *Foxp3* gene [123]. Previous studies in mice showed that ablation of *Stat3* in the hematopoietic system using the *Mx1-Cre-loxP* system was accompanied by a reduction in the number of tumor-infiltrating Tregs [124]. We have reported that a small molecular inhibitor of the STAT-3 pathway, WP1066, inhibited the induction of Foxp3 expression in peripheral T cells and downregulated Foxp3 expression in nTregs [64,65] and that this likely accounted for the marked *in vivo* antitumor effects and enhancement of cytotoxic T cell responses. Additionally, we reported that STAT-3 blockade had negligible inhibitory effects on effector T cell cytotoxicity (which is mediated by the STAT-5 activation pathway), which may compromise immunological tumor clearance. In comparison, many of the other types of anti-Treg agents (Ontak, anti-CD25 antibodies, CTLA-4--blocking agents, etc.) are cross-reactive with other T cell populations that exert effector responses.

Despite recent advancements in immunotherapies for malignant gliomas, the overall prognosis for individuals with these tumors remains very poor. Given the cellular complexity of this very aggressive tumor, any one treatment modality alone is unlikely to be effective against it. Ultimately, improving outcomes in patients with malignant gliomas will require a multifaceted treatment approach combining various treatment modalities. Given the multiplicity of immunosuppressive mechanisms, anti-Treg agents may be most beneficial in patients whose immunosuppression is dependent on this mechanism (i.e., elevated number or fraction of CD4⁺/Foxp3⁺ Tregs in the peripheral blood and/or marked intratumoral infiltration with Tregs). Future studies of Treg inhibition/modulation in combination with other immunotherapeutics, such as IFN- α , IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), monoclonal antibodies against tumor antigens, peptides, DCs, and anticancer vaccines, would present an opportunity to further potentiate these agents' therapeutic efficacy, and not just for malignant gliomas. The recent success of several clinical trials of immunotherapy for glioma patients further stresses the need for development of promising adjunct treatments, especially combination treatments, of malignant gliomas. However, these and other future developments must be integrated into a comprehensive treatment program incorporating current treatment modalities.

Acknowledgments

This work was supported by The Dr. Marnie Rose Foundation, the Anthony D. Bullock III Foundation, an institutional research grant from The University of Texas M. D. Anderson Cancer Center, and National Institutes of Health grants CA120813-01 and A177225-01 (to A.B.H.).

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Fig. 1.

Schematic illustration of Treg development. (A) Illustration of the development of nTregs. nTregs are selected with an intermediate affinity for the MHC II/self-peptide complex in the thymus with an intermediate affinity for the MHC II/self-peptide complex. They then enter the peripheral circulation as CD4⁺/CD25⁺/Foxp3⁺ cells. The nTregs exert their effect on immune effector cells primarily via cell-to-cell contact both peripherally and within the tumor microenvironment. (B) Illustration of the development of iTregs. Tr1 and Th3 cells fall under the rubric of iTregs and are also derived from the thymus; however, they enter the peripheral circulation as naïve (CD4⁺/CD25⁻/Foxp3⁻) T cells. They are then induced in the periphery to differentiate into regulatory T cells via a myriad of redundant pathways emanating from the tumor microenvironment. Differentiation of naïve CD4⁺ cells into Tr1 and Th3 cells is cytokine-dependent, as is the mechanism by which the iTregs exert their effects on immune effector cells.

Fig. 2. Immunohistochemical staining of human tissue sections for Foxp3 in (A) oligodendrogloma, (B) mixed oligoastrocytoma, (C) anaplastic oligodendrogloma, (D) anaplastic mixed oligodendrogloma, (E) low-grade astrocytoma, (F) anaplastic astrocytoma, (G) glioblastoma, and (H) gliosarcoma. Tissue sections for Foxp3 show faint staining with few nonclustering Tregs (B, D, F). The Treg number increased as the tumor grade increased (E-G), although they were nearly absent from tumors with oligo-based histologies (A and C). All of the images were taken at a magnification of 400x.