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Several immunological approaches to treating malignant glioma deserve further study.

Immunotherapeutic Strategies for Malignant Glioma

Robert A. Fenstermaker, MD, and Michael J. Ciesielski, PhD

Background: *Despite advances in surgery, radiation therapy, and chemotherapy, only modest improvement has been achieved in the survival of patients with malignant gliomas.*

Methods: *The authors review the immunologic aspects of gliomas, potential targets for therapy, and issues surrounding current immunotherapeutic strategies directed against malignant gliomas.*

Results: *The blood-brain barrier and the purported immunological privilege of the brain are not necessarily insurmountable obstacles to effective immunotherapy for brain tumors. Preclinical studies suggest a number of potential therapeutic avenues. Translational studies offer the prospect of providing substantial new information about immunological trafficking in the nervous system and suggesting the most fruitful approaches to immunotherapy for malignant gliomas.*

Conclusions: *More effective adjuvant treatments for malignant gliomas are needed. The applicability of immunological approaches in the treatment of these tumors warrants continued study.*

From the Departments of Neurosurgery (RAF, MJC) and Immunology (MJC), Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York.

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Address reprint requests to Robert A. Fenstermaker, MD, Roswell Park Cancer Institute, Department of Neurosurgery, Elm and Carlton Streets, Buffalo, NY 14263. E-mail: robert.fenstermaker@roswellpark.org

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Introduction

Malignant gliomas are the most common brain tumors in adults and are responsible for approximately 15,000 cancer deaths in the United States each year.¹ The incidence of the most common type of malignant glioma, known as glioblastoma multiforme (GM), is increasing, particularly in older age groups.^{2,3} Without treatment, the median survival for patients with GM is 12 to 16 weeks. Younger patients and those with higher performance status and minimal neurologic deficit generally survive longer. With conventional cancer treatments including surgery, radiation therapy, and chemotherapy, survival is 12 to 24

months for patients with more favorable prognostic factors and 6 to 9 months for those with poorer factors.⁴ The completeness of surgical resection may also affect the time to recurrence and overall survival, although this remains a controversial question.⁵

Further advances in surgical techniques are not likely to markedly improve survival since even complete resection of malignant gliomas, as defined by immediate post-operative magnetic resonance imaging, almost invariably leaves residual microscopic disease.⁶ Residual tumor cells may grow within adjacent, potentially functional, brain tissue. With tumor recurrence or progression, those who are candidates for reoperation, brachytherapy, stereotactic radiosurgery, or further chemotherapy with second-line agents may obtain a second or third remission. Such remissions or stabilizations of neurologic function tend to be brief, with eventual decline in neurologic function and death. Moreover, older patients and those with poorer performance status may benefit less from further treatment or may be ineligible for reoperation due to severe neurologic disability or poor general clinical condition. New and more effective adjunctive treatments are needed, particularly for initial treatment of residual microscopic disease following surgery.

On rare occasions, metastatic tumors will spontaneously regress following resection of a primary renal cell tumor. This observation and others like it have led to the inference that immunological processes can influence the course of malignant tumors. Recent advances in immunotherapy for melanoma, renal cell carcinoma, and other solid tumors have generated a resurgence of interest in immunological approaches to combat malignant gliomas.⁷ This has been aided by improvements in our understanding of the role of local immunity in the central nervous system (CNS) and identification of certain brain tumor-specific antigens for direct molecular targeting. While immunological approaches to malignant brain tumors have not progressed as far as those for either melanoma or renal cell carcinoma, a growing body of information points to the applicability of these methods to the challenging problem of CNS tumors including gliomas.

Immunological Privilege of the Brain

Traditionally, the brain has been regarded as an immunologically privileged organ site since it possesses a distinct blood-brain barrier (BBB) and lacks discrete lymphatic structures.⁸ Moreover, engraftment of tumors and other tissue tends to be more successful in the brain than in other organs.⁹ For some time, the apparent absence of unactivated T lymphocytes in the brain was thought to imply a deficiency in the afferent limb of the immune response. It has been hypothesized that these factors allow malignant gliomas to evade immune surveillance. It has also meant that expectations for the success of

immunologically based antitumor strategies against malignant brain tumors have been guarded.

A large body of evidence now supports a modern view that calls these assumptions into question. For example, it was observed that when human albumin is injected into the ventricles of rats, specific antibodies appear in both serum and lymph.^{10,11} This phenomenon is attenuated by cervical lymph node obstruction. Similarly, injection of radiolabeled protein into the brains of rabbits leads to the accumulation of tracer in cervical lymph nodes.¹² This observation suggests that trafficking does occur between immunologically competent cells in the brain and peripheral elements of the immune system. In animals, and probably in humans, the initial peripheral immune response occurs mainly in the cervical lymph nodes.¹³⁻¹⁵

There is a growing body of data to support the existence of both afferent and efferent immune pathways in the CNS. Evidence suggests that antigen presentation cells (APCs) are indeed present in the brain.¹⁶ Microglial cells express a number of macrophage-associated markers and major histocompatibility (MHC) antigens suggesting that they may actually function as APCs in the brain. This is further supported by the observation that glioma cells genetically modified to express interferon gamma (IFN- γ) and introduced into the brain cause direct priming of microglia with subsequent tumor rejection which is accompanied by CD4⁺ and CD8⁺ tumor cell infiltration.¹⁷ While the exact site and mechanism of antigen presentation by APCs in the CNS are not yet known, it appears that the brain is capable of producing an antitumor immune response via resident and regional APCs.

The presence of tumor infiltrating lymphocytes (TILs) in malignant gliomas suggests the possibility that specific immune effector cells may be capable of invading such tumors. Infiltrating lymphocytes within human gliomas have been reported to be associated with longer survival.¹⁸ Animal studies clearly show that tumor-specific lymphocytes can infiltrate gliomas. For example, in the GL261 mouse glioma model, peripherally produced immune effector cells enter brain tumors following stimulation of bone marrow dendritic cells with tumor-derived RNA and interleukin 12 (IL-12).¹⁹ Infiltrates of CD4⁺ and CD8⁺ T cells are found in the GL261 tumors implying that specific TILs are produced peripherally and then traffic into CNS tumors. In addition to antitumor T lymphocytes trafficking into the CNS, antigen presentation in the brain leads to the recruitment of B cells with specific antibody synthesis.²⁰

TILs harvested from human malignant gliomas have been shown to have considerably greater cytolytic effects against autologous glioma cells than allogeneic glioma cells in culture.²¹ This observation has led to at least one clinical trial of intracavitary TIL immunotherapy combined with IL-2.²² In this study, TILs were harvested from malignant gliomas and cultured with IL-2. After reinjection into the tumor cavity, 2 of 6 patients experienced partial

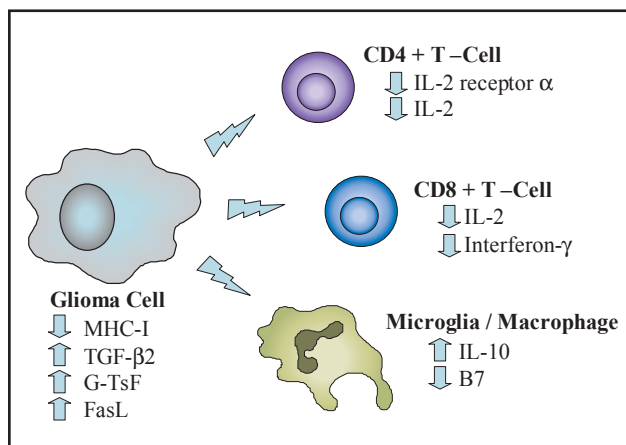


Fig 1. — Immunosuppression by gliomas. Glioma cells secrete a number of different immunosuppressive mediators that inhibit antitumor immunity in humans and rodents (IL = interleukin, G-TsF = glioma-derived T-cell suppressor factor, TGF- β = transforming growth factor beta).

responses and 1 achieved a complete response. Thus, TIL immunotherapy offers one prospect for glioma treatment.

Many therapeutic failures have been blamed on the presence of an intact BBB, behind which tumor cells remain isolated from chemotherapeutic and immunological attack. The situation with gliomas may be somewhat more complex, however. While the integrity of the BBB is preserved during early tumor growth, evidence suggests that as a cerebral glioma progresses, lymphocyte infiltration and MHC class II expression both parallel that of gliomas grown in the subcutaneous space.¹⁶ Thus, integrity of the BBB and accessibility to therapeutic targeting are subject to change as a tumor develops.

Suppressive Effects of Malignant Gliomas on the Immune System

Malignant glioma cells secrete a variety of substances that suppress antitumor immunity in humans (Fig 1). Glioma patients often fail to exhibit delayed skin hypersensitivity reactions and are frequently anergic at the time of tumor diagnosis.^{23,24} Following surgical removal of a glioma, systemic T-cell responsiveness is at least partially restored. However, T-cell function once again declines with the onset of tumor recurrence.²⁴ Much of this glioma-derived immunosuppression is associated with transforming growth factor-beta 2 (TGF- β 2) secreted by the tumor, accompanied to a degree by TGF- β 1 and TGF- β 3. Down-regulation of TGF- β expression by antisense methodologies in rat 9L glioma cells enhances tumor cell immunogenicity, prolongs survival, and can lead to tumor eradication.²⁵

Another soluble factor is known as glioma-derived T-cell suppressive factor (G-

TsF). While this molecule has not been fully characterized, it is probably closely related to TGF- β 2.²⁶ Glioblastoma cell lines produce G-TsF, which inhibits proliferation and IL-2 production by T cells derived from healthy individuals.²⁷ Progression of malignant gliomas is accompanied by other defects in local and systemic tumor immunity as well. These include decreased expression of IL-12, IFN- γ , and TNF- α , as well as increased expression of IL-4, IL-5, IL-6, and IL-10.²⁸ MHC class II expression has also been shown to be down-regulated by IL-10 in human and murine systems.^{29,30} Expression of Fas and Fas ligand (Fig 2) has also been noted in microglia and in glioma cells where they probably contribute to immunosuppression as well.^{31,32} Similarly, the co-stimulatory molecule CD80 (ie, B7.1) is down-regulated by glioma cells.²³ Together, these alterations probably exert important regulatory effects on both local and systemic cellular immune function and may be responsible for the observation of apoptosis and anergy of TILs in malignant gliomas.^{33,34} This failure of T cells within gliomas may be more confined to CD4⁺ than to CD8⁺ cells.³⁵

T cells from glioma patients have impaired responses to antigens and T-cell mitogens with reduced proliferation and IL-2 production.³⁶ In addition, CD8⁺ T cells from glioma patients have low levels of CD28 co-stimulatory molecule, defective IL-2 α receptor subunit expression, and reduced phosphorylation of CD3 ζ T cell-antigen receptor chains.³⁷⁻³⁹ Certain other members of the IL-2 cytokine family (IL-7, IL-9, and IL-15) and their cognate IL-2 receptor subunits (IL-2R β , IL-2R γ , IL-7R α , IL-9R α , and IL-15R α) are also down-regulated.⁴⁰ In combination, the effects summarized here lead to a profoundly immunocompromised state at the time of initial tumor diagnosis and upon tumor recurrence after first remission. Thus, the expression of immunosuppressive factors by an established glioma can be expected to limit the extent of any immunological

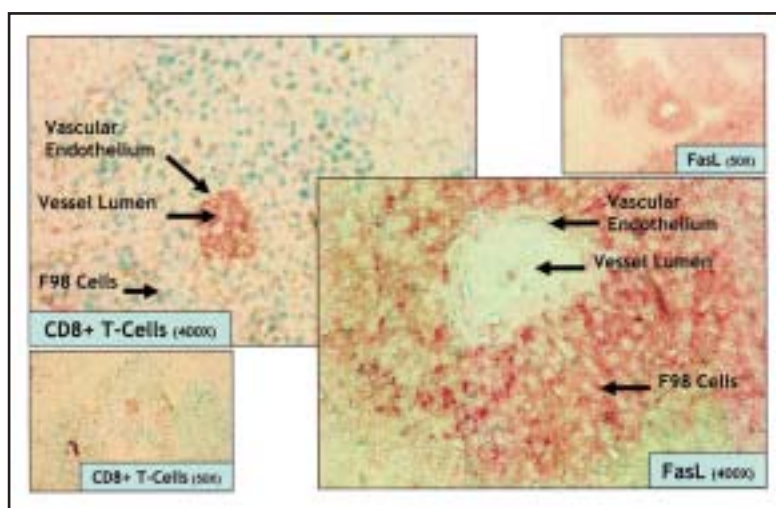


Fig 2. — Fas ligand expression in gliomas. Immunostaining of rat F98 glioma tissue for Fas ligand (purple) shows strong expression in tumor cells surrounding blood vessels. CD8⁺ cells, which are known to express apoptosis-inducing Fas receptor (also in purple), are present in the vessel lumen and are not found infiltrating the tumor. The tissue is counterstained with methyl green.

response to tumor vaccines or inhibit the action of other cell-mediated immune strategies. If levels of TGF- β 2 and other immunosuppressive factors can be reduced by glioma resection and the effects of residual microscopic disease counteracted in some way, a window of opportunity might be created to deliver antitumor immunological therapies. Similar strategies have been reported with nephrectomy for renal cell carcinoma in advance of IL-2-based immunotherapy.⁴¹ Therefore, much as surgery is thought to create an optimal environment within which radiation therapy and chemotherapy can be most effective, maximal safe surgical resection may enable immunotherapy to be more effective. The success of immunotherapy for malignant brain tumors will require concomitant efforts to mitigate the immunosuppressive effects produced by the tumor itself. At present, the most effective such method appears to be surgical resection or debulking.

Tumor-Specific Antigens as Targets for Immunotherapy

Expression of MHC molecules by glioma cells is essential for the correct presentation of tumor-specific antigens to generate a potent antitumor immune response. The effectiveness of antigen presentation is dependent on the affinity of tumor antigens for the particular MHC molecules expressed by the glioma cells of each individual patient. Among other factors, this requirement leads to considerable variability of responsiveness when carrying forward preclinical findings to human trials. MHC class I antigen expression on tumor cells is required for immune effector cells to produce CD8 T-cell recognition and lytic action. Similarly, MHC class II expression is required for induction of humoral responses and CD4 T-cell helper function. Therefore, lack of MHC expression on tumor cells can lead to immunological escape.

Central to the search for effective immunotherapy for malignant brain tumors is the identification of immunoreactive brain tumor antigens that are distinct from those present on normal brain tissues. A number of potential antigens have been identified for use in immune-based strategies, although many are found in normal tissues as well. In some cases, as with wild-type epidermal growth factor receptor (EGFR), the difference is quantitative rather than qualitative. Unique molecular targets without close similarity to molecules present in normal tissues should provide the best targets for immunotherapy.

A number of potentially targetable antigens have been identified in brain tumors. Tenascin, for one, has been studied extensively as a target for direct immune attack via specific antibodies.⁴² Tenascin is a large molecule that is a component of the extracellular matrix where it mediates the interaction between neurons and glia. It is expressed at high levels in the extracellular matrix of malignant gliomas.⁴³ Another tumor-specific marker found in brain

and renal tumors is SART-1, which was originally described in squamous cell carcinoma and is similar to the HLA-restricted MART antigens used in melanoma vaccine trials.^{44,45} SART-1 peptides bind to HLA-A24, binding motifs and inducing HLA-A24-restricted T lymphocytes.^{45,46} Similarly, SART-3 tumor rejection antigen has also been noted in brain tumors and may serve as a useful target for peptide immunization.⁴⁷ The antigen 4Ff2, which is highly expressed on the surface of mitotically active endothelial cells and tumor cells including gliomas, is of interest for specific targeting.⁴⁸

One notable tumor-specific antigen present in malignant gliomas is the EGFR variant III (EGFRvIII) protein. This protein has been the subject of a number of studies concerning various immune-based methods for glioma therapy. In addition to malignant gliomas, EGFRvIII has been identified in tumors of the breast, ovary, prostate, and lung, making the prospect for specific antitumor immunotherapy using this particular antigen of broad potential interest.⁴⁹⁻⁵¹

Clinical Immunotherapy Trials for Glioma

Initial efforts at tumor vaccine development have generally focused on the production of cytotoxic T lymphocytes (CTLs) with antitumor specificity following immunization. A second and more difficult task is to determine whether a clinical antitumor effect can be detected. A number of phase I and phase II trials have been conducted to investigate various immunological approaches to glioma therapy. At present, however, no specific vaccines are commercially available for this application.

Early efforts in the field of immunotherapy for gliomas involved active immunization with such agents as bacille Calmette-Guérin (BCG).⁵² A more recent study combined BCG with the patients' own irradiated tumor cells. CD4⁺ and CD8⁺ cells were isolated from peripheral blood, stimulated in culture, and readministered to the patients with low toxicity.⁵³

Other clinical trials have been conducted using a biological response modifier derived from the *Serratia marcescens* bacterium (ImuVert), which augments natural killer (NK) cell activity.⁵⁴ One clinical trial performed in patients with recurrent malignant gliomas revealed a response rate of 16%.⁵⁵ In another study of ImuVert and concurrent radiation therapy, some toxicity in the form of hypotension was encountered, with survival similar to that of standard therapy in historical controls.⁵⁶ Along similar lines, immunization with a killed tumor cell vaccine modified with Newcastle disease virus has been reported to have an effect on tumors. One study of glioma patients treated in this manner revealed no difference in survival compared to those patients treated with standard therapy, although a strong peripheral antitumor immune response was documented.⁵⁷

Interleukin 2 and Granulocyte-Macrophage Colony-Stimulating Factor

IL-2 is an important cytokine capable of stimulating effector T-cell maturation. High doses of systemically administered IL-2 are required to achieve effective levels in the brain for immunotherapy against brain tumors.⁵⁸ Doses at these levels may produce toxic side effects that limit the utility of any such approach. Several alternatives have been investigated to circumvent this problem. One approach has employed low-dose IL-2 in combination with lymphokine-activated killer (LAK) cells introduced on a direct basis into the tumor resection cavity or the surrounding brain tissue.^{59,63} Although neurotoxicity has been noted with some of these regimens, good clinical responses and some long-term survivors have also been reported.^{60,62} Jeffes et al⁶⁴ used IL-2 and phytohemagglutinin (PHA) to activate peripheral blood lymphocytes prior to direct intracavitary lymphocyte injection. While *in vitro* antitumor activity was enhanced in this study, clinical antitumor responses were not dramatic. In contrast to these studies, Barba et al⁶⁵ reported significant neurologic complications in patients treated with intracavitary IL-2 and LAK cells.

Combination therapy with IL-2 administered intracranially and systemic IFN- α has also been studied in a dose-escalation format.⁶⁶ At higher doses of intracavitary IL-2, cerebral edema was frequently noted with focal neurologic symptoms. While dexamethasone reduced these adverse effects, it also appeared to attenuate the antitumor activity induced by IL-2 *in vitro*.⁶⁷ Moreover, 10% of patients experienced sterile abscesses in the tumor resection cavity, requiring surgical decompression.⁶⁸

Sobol et al⁶⁹ described a clinical immunization trial using autologous glioma cells modified by gene transfection to express IL-2. In this study involving a single patient, CD8⁺ T cells were produced in the patient's peripheral blood, and tumor necrosis was observed as well. So far, a larger series of patients treated in this fashion has not yet been reported. However, animal studies have suggested better therapeutic effect with this type of approach than with systemic IL-2 administration.^{70,71}

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is another potent antitumor agent with the ability to stimulate a CTL response. GM-CSF induces the recruitment of APCs and may activate antigen presentation pathways for MHC class I-mediated processes. GM-CSF has also been shown to stimulate intracranial responses to irradiated tumor cell vaccines.⁷²⁻⁷⁴ Continuous infusion of GM-CSF, in combination with intermittent injection of irradiated glioma cells, has also been found to induce complete regression of intracranial tumors in rats.⁷⁵ To date, clinical studies with GM-CSF as an adjuvant to immunotherapy for gliomas are relatively limited. Adoptive immunotherapy has been attempted using lymphocytes derived from the lymph nodes of patients with gliomas.

Harvested cells were stimulated in culture with GM-CSF and IL-2 and then reinfused into patients. Several responses and long-term survivors were reported.⁷⁶ Thus, GM-CSF may serve as a useful vaccine adjuvant with limited toxicity in human clinical trials of anti glioma vaccine therapy.

Dendritic Cells

Preclinical studies on dendritic cell vaccine techniques have been encouraging, and the more recent introduction of these methods into the clinic offers an important new avenue for investigation.^{77,78} One laboratory study by Ni et al⁷⁷ compared pulsed and nonpulsed dendritic cells against the mouse GL261 glioma cell line. Animals that received dendritic cells pulsed with killed GL261 cells displayed enhanced survival, strong allogeneic responses and resistance to further tumor challenges. Another study of dendritic cells pulsed with antigens eluted from 9L rat gliosarcoma cells showed prolonged survival and infiltration of tumors with CD4⁺ and CD8⁺ cells.⁷⁹

Kikuchi et al⁸⁰ isolated dendritic cells from the blood of patients and fused them with autologous glioma cells from individual patients. Fusion cells were injected intradermally in proximity to cervical lymph nodes, and IFN- γ expression was subsequently detected in the peripheral blood monocytes of some patients. While no clear antitumor response was shown, the safety of the technique was confirmed.⁸⁰ Another study of mature dendritic cells cultured with autologous glioma cellular lysate showed a strong induction of CTL activity and potentiation of allogeneic T-cell proliferation.⁸¹ Yu et al⁷⁸ pulsed dendritic cells with peptides eluted from the surface of malignant glioma cells and reinjected them intradermally. Cytotoxic activity was detected in most patients combined with infiltration of the tumors by CTLs. Studies also point to the relative safety of locoregional therapy, which might help to circumvent problems posed by a partially intact BBB. Importantly, none of these studies reported evidence of autoimmune neurotoxicity.⁸²

Epidermal Growth Factor Receptor Mutants

Amplification and overexpression of the EGFR gene are frequently seen in malignant gliomas.^{83,84} In addition to overexpression of the wild-type EGFR, some gliomas express mutant EGFR species arising from specific EGFR gene rearrangements (Fig 3).⁸⁵⁻⁸⁹ These EGFR mutants are constitutively autophosphorylated and oncogenic, and they contain deletion or duplication of specific sets of exons corresponding in some cases to entire functional domains of the protein.

EGFRvIII is the most common EGFR deletion mutant and is expressed in over 50% of malignant gliomas.⁹¹ The

EGFRvIII gene contains deletion of part of the extracellular portion of the molecule creating a novel "fusion" junction. This mutant EGFRvIII gene is probably created by aberrant recombination between EGFR introns, and it encodes a 140–145 kd receptor with a unique antigenic tumor-specific epitope at the site of the fusion junction. EGFRvIII is of particular interest because it is a true tumor-specific antigen that does not occur in normal tissues. In contrast, the wild-type EGFR is also found in normal tissue such as liver and skin and to a lesser extent in normal astrocytes. Therefore, effective and specific targeting of EGFRvIII should cause little collateral injury to normal tissue carrying the wild-type EGFR.

Other EGFR mutations have been identified in malignant gliomas, including those produced by tandem duplication, rather than deletion, of EGFR gene sequences.^{88,89,92} A number of these EGFR variants have been characterized in human glioma cell lines and tumors removed at surgery. In each case, evidence strongly supports the notion that these mutant genes arise by aberrant recombination between introns in the EGFR gene. Together, these mutants identified to date represent a distinct class of EGFR mutants with potent oncogenic potential due to constitutive receptor activation. The mutant EGFRs are of interest because each contains its own unique epitopes that may permit specific immunological targeting.⁹³

Tumor-Specific Targets in Gliomas

Several clinical trials have been performed using antibody therapy for malignant gliomas. Tenascin has been used as a target for antibody-based immunotherapy including direct intracavitary application of radiolabeled anti-tenascin antibody.⁹⁴⁻⁹⁸ In a phase I study of 42 patients with newly diagnosed glioblastoma, dose-limiting toxicity was identified at 120 mCi of ¹³¹I-labeled 81C6 monoclonal anti-tenascin antibody.⁹⁸ Median survival for patients with GM was 69 weeks with a low incidence of symptomatic radiation necrosis. Another phase I study by Riva et al⁹⁷ utilized the mouse monoclonal antibody BC-4 labeled with ⁹⁰Y as the radioactive conjugate in patients with prior extensive treatment for GM. In this study, the maximum tolerated dose was 25 mCi. No clinical responses were noted, although in contrast to the study with ¹³¹I-labeled 81C6 monoclonal anti-tenascin, all patients had advanced disease and had failed other conventional treatment regimens. Paganelli et al⁹⁴ employed a variation of this technique in their treatment of 24 patients with malignant gliomas, 16 of whom had GM. Overall, 25% of patients had objective responses with stable disease in 50%. Little toxicity was observed at doses up to 20 mCi. Reardon et al⁹⁵ reported on a follow-up phase II study of the ¹³¹I-labeled 81C6 monoclonal anti-tenascin antibody in 33 patients

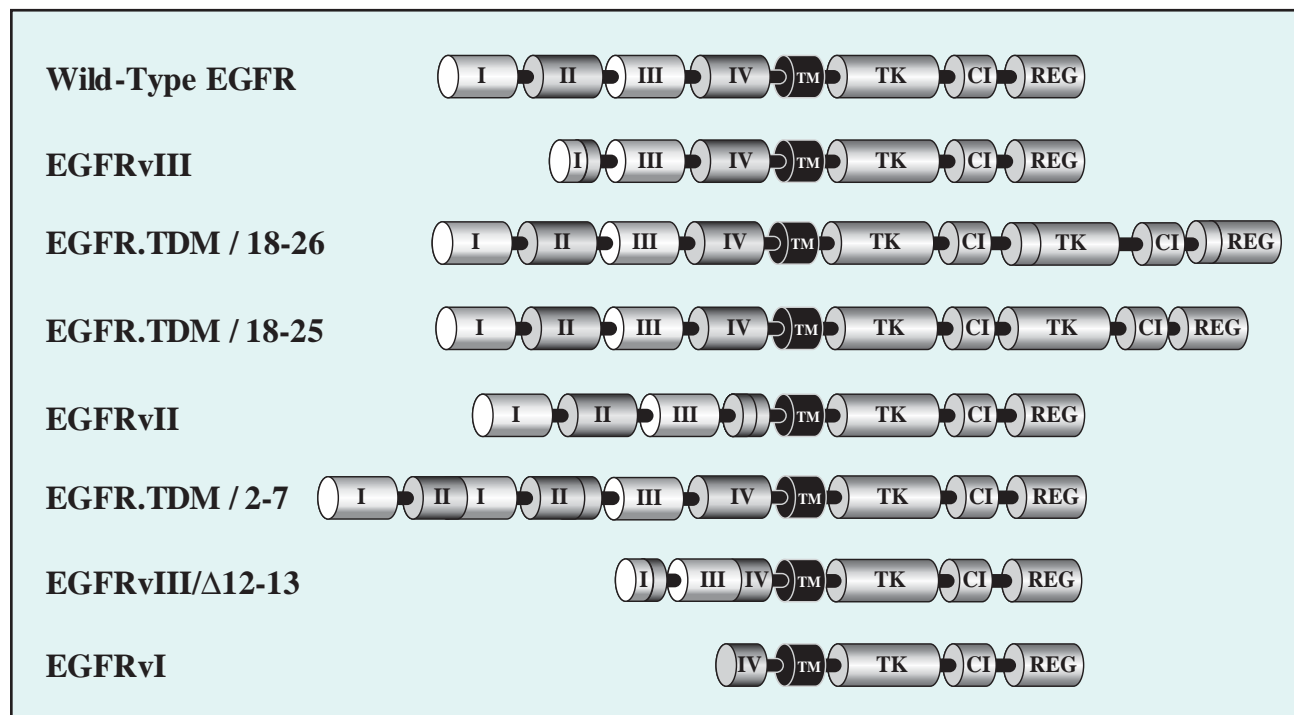


Fig 3. — Structure of epidermal growth factor receptor (EGFR) and its recombination mutants. The domain structure of wild-type EGFR is based on the exon structure as defined by Callaghan et al.⁹⁰ Malignant gliomas have a number of mutants that arise from gene recombination and novel tumor-specific epitopes that are of potential value for immunological targeting. The gene that encodes the EGFRvIII molecule contains a deletion of exons 2 through 7. This creates an oncogenic receptor with a novel amino acid sequence. EGFRvIII is the most common of the various recombination mutants, occurring in over 50% of high-grade gliomas.⁵¹ EGFR mutants that are less common have deletion, or in some cases tandem duplication of internal sequences, that produce their own unique epitopes. So far, immunological targeting has focused on EGFRvIII, but the other rarer EGFR mutants are potential targets as well (I, II, III, and IV = extracellular domains, TM = transmembrane domain, TK = tyrosine kinase domain, CI = calcium-mediated internalization domain, REG = regulatory domain).

with newly diagnosed malignant gliomas. Median survival for the patients with glioblastoma was 79.4 weeks, which was stated to be better than historical controls. While survival was not dramatically enhanced in these studies, the incidence of radiation necrosis and both systemic and neurologic toxicities was low.

Other tumor-specific targets offer prospects for antibody-mediated therapy as well. The human monoclonal antibody ACA-11, which reacts against tumor antigen (TA226) found in malignant gliomas but not in normal brain tissue, produces antibody-dependent cell-mediated cytotoxicity (ADCC).⁹⁹ In a phase II study of ACA-11 performed in patients with gliomas, the antibody was given intermittently for 24 weeks. A partial response rate of 24% was reported.

The tumor antigen that is recognized by the monoclonal antibody CLN-IgG is located on the outer surface of the cell membrane of gliomas and other tumors but not in normal brain tissue. Like ACA-11, CLN-IgG is capable of producing ADCC. A small phase II study was conducted with CLN-IgG in 10 patients with malignant brain tumors. CLN-IgG was administered to 6 patients with glioblastoma, 1 with medulloblastoma and 3 with brain stem glioma.¹⁰⁰ Two patients had significant decreases in tumor size, and 1 had stable disease by imaging criteria.

In contrast to these antigenic targets, which may not be so precisely defined, a potentially targetable and well-characterized IL-13 receptor has been clearly linked to malignant glioma progression.¹⁰¹ The glioma-specific form of IL-13 receptor is targetable by immunotoxins and potentially by other immunologically mediated methods as well.^{102,103} Expression in gliomas is reported to be highly consistent and therefore potentially widely applicable for treatment applications.¹⁰¹

EGFR is one of the most consistently expressed cell-surface molecules on malignant gliomas. A number of antibodies to the human wild-type EGFR have been developed including chimerized/human monoclonals.¹⁰⁴ A fully human anti-EGFR antibody E7.6.3 has also been developed that completely blocks the receptor leading to inhibition of downstream signaling and internalization, with resulting apoptosis.¹⁰⁵ Results in animals appear promising, even without concomitant chemotherapy.

EGFR carries the distinct disadvantage of fairly ubiquitous expression. However, for immune-mediated attack, a truly tumor-specific target is preferred. Consequently, EGFRvIII carries significant potential as a specific antitumor target. Several anti-EGFRvIII antibodies, including L8A4, Y10, and 806, have been developed for diagnostic and therapeutic applications.¹⁰⁶⁻¹⁰⁸ These antibodies have shown inhibition of tumor growth and necrosis of xenograft tumors grown in nude mice.¹⁰⁸

If a tumor antigen is seen as *self*, it may not generate an immune response. The development of anti-idiotypic antibody vaccines, like Y10, is of interest in this regard because such antibodies are capable of inducing both

humoral and cellular immune responses even when the host is anergic to immunization by the tumor antigen itself. An anti-idiotypic antibody is a mirror image of the target antigen. When used as a vaccine, the anti-idiotypic antibody generates an immune response capable of recognizing the tumor-specific antigen.

In addition to antibody-mediated approaches, EGFRvIII is an appropriate target for tumor vaccine strategies. EGFRvIII peptide vaccines have been studied in rodent melanoma models and recently, one such vaccine has been shown to be effective in an intracranial mouse melanoma model.^{109,110} These types of vaccines induce specific cellular immune responses against tumors and act in part via an ADCC-mediated mechanism.¹¹⁰

Cellular Antiglioma Immune Response Induced by a Survivin DNA Vaccine

Intracellular proteins are also targets for immunologically mediated attack. Epitopes of intracellular proteins are found at the cell surface of APCs in association with MHC I molecules. If recognized by T cells, these molecules can induce strong and effective responses against tumor cells bearing the same MHC-I-associated epitopes. The cellular protein survivin is a member of the family of proteins known as inhibitors of apoptosis proteins (IAPs). Human gliomas and other tumors express survivin at high levels, whereas normal cells do not.¹¹¹ Therefore, survivin represents a unique type of tumor-specific target for immunologically based therapy. Since survivin is an intracellular protein that is expressed only in tumor cells, it may be possible to produce an MHC-I-restricted cellular immunological attack in response to a survivin vaccine.¹¹² Schmitz et al¹¹³ showed that survivin can induce CD8⁺ T-cell immune responses in vitro when presented by dendritic cells. Recombinant survivin incubated with dendritic cells can induce specific MHC class I-restricted CTLs.

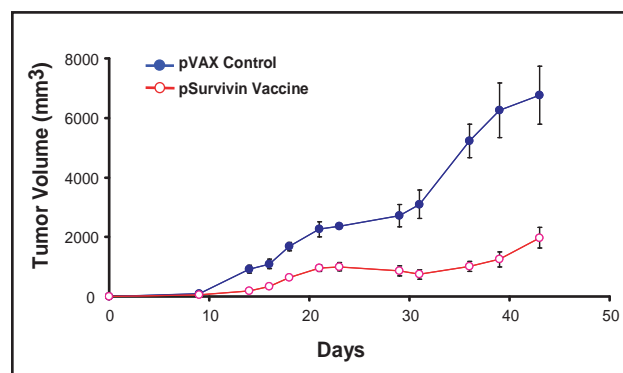


Fig 4. — Growth of syngeneic F98 glioma cells in Fischer rats. A single dose (10 μ g) of DNA vaccine (designated pSurvivin) containing a truncated survivin gene or vaccine vector without survivin (pVAX) was given subcutaneously 4 days after injection of 1×10^6 tumor cells. GM-CSF (50 ng) and was added as a vaccine adjuvant to each injection. Tumor volumes were calculated from serial tumor diameter measurements (values \pm SEM, $n = 7$ rats per group).

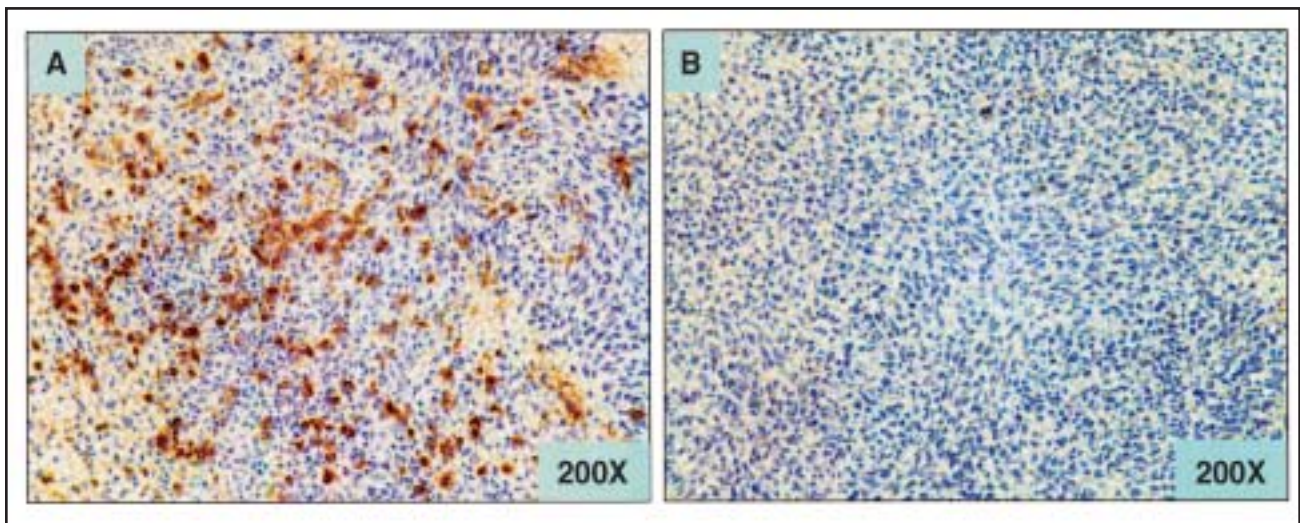


Fig 5. — CD8⁺ infiltration of F98 gliomas following vaccination. CD8⁺ cells in F98 gliomas were obtained 45 days after tumor cell implantation (41 days after vaccination) with truncated survivin gene (pSurvivin, left) or vector alone (pVAX, right). CD8⁺ cellular infiltrates were detected by primary antibody and DAB staining (brown) with hematoxylin counterstain.

The results of an experiment in which F98 glioma cells, which express survivin at high levels, were injected into syngeneic Fischer rats are shown in Fig 4. Four days after tumor implantation, rats were vaccinated with either a DNA vaccine vector alone (pVax) or the same vector containing a truncated human survivin gene (pSurvivin). Each vaccine included GM-CSF as a vaccine adjuvant. By itself, GM-CSF had no significant effect on growth of F98 tumors in Fischer rats (data not shown). The DNA vaccine vector without survivin had no effect on tumor growth; however, a single dose of pSurvivin attenuated tumor growth. Immunohistochemical examination identified large numbers of CD8⁺ cells in the tumors of rats vaccinated with pSurvivin plus GM-CSF (Fig 5). In contrast, few CD8⁺ cells were identified in the tumors of rats injected with the DNA vaccine vector alone. These findings are consistent with the production of a specific cellular anti-tumor immune response against F98 gliomas.

Vaccination Strategies

Peptide vaccines have the advantage of presenting distinct epitopes that do not require host cellular machinery for their production. They are limited in terms of the number and context of epitopes that may be presented at any one time. DNA vaccines have a number of potential advantages over peptide vaccines in particular. DNA vaccines consist of a genetically engineered plasmid vector containing a portion of the gene that encodes a tumor-specific target antigen. While chemically synthesized peptides are of relatively small size, the proteins generated from DNA vaccine vectors are potentially of much greater size. The proteins that they produce may undergo protein processing with the addition of carbohydrates and proper folding to create a native conforma-

tional structure that mimics the target antigen. Consequently, the number of potential epitopes presented by the antigen and their specificity may be replicated better, leading to the production of more “relevant” antibodies and a more effective cellular immune response. DNA vaccines may produce more robust cellular immune responses than peptides, since proteins encoded by vaccine vectors are produced intracellularly. Therefore, proteolytic cleavage and presentation within the specific context of MHC class I antigens may be more likely to occur than with direct vaccination using peptides. Also, peptide vaccination may require relatively large quantities of material with more complicated production, storage, and handling than DNA.

Immune tolerance is a potential obstacle for any immunotherapeutic strategy that relies on vaccination against specific antigens. The immune system is tolerant to many *self* antigens. The addition of cytokines or other immune modulators to vaccination protocols may help to break this tolerance. One immunological antitumor strategy involves the induction of autoreactive T cells to murine gp100 in mice using human gp100 xenoimmunization.¹¹⁴ This approach has been used as the basis for xenoimmunization in human clinical trials to treat malignant melanoma. A similar approach could be used with other xenogeneic glioma-specific antigens to break immune tolerance to the human epitopes. In this particular vaccine strategy, the homologous epitopes encoded by the xenogeneic DNA may act as molecular mimics recognized as *non-self*, resulting in an immune response against host *self* gene products.

Gene therapy studies in animals have suggested another strategy for immunologically mediated attack on gliomas. Gene therapy using the herpes simplex virus thymidine kinase (HSVtk) gene in combination with ganciclovir has undergone extensive evaluation for the direct

treatment of malignant gliomas. The effect on the tumor of the HSVtk ganciclovir is partially dependent on a local bystander effect with metabolic coupling of adjacent tumor cells. In addition, Okada et al¹¹⁵ have documented a distant bystander effect mediated by CTLs that provides lasting antitumor immunity. Thus, the diverse effects of HSVtk gene therapy provide yet another paradigm for evaluating immunological strategies for treatment of malignant gliomas.

Conclusions

Recent years have brought an improved understanding of both afferent and efferent limbs of the immune response to antigens presented in the CNS. A more detailed picture of immune suppression by gliomas and potential glioma-specific targets is also emerging. Although clinical trials of immunotherapy for gliomas are relatively limited, advances with other malignancies and data from rodent brain tumor models reveal a justification for further study. Future approaches are likely to focus on preclinical studies and therapeutic trials involving immunization with anti-idiotypic antibodies, DNA and peptide vaccines, and dendritic cell strategies. Ultimately, immunity against a single tumor-specific antigen may be insufficient to prevent tumor growth or eradicate an established neoplasm. Thus, vaccines directed against multiple tumor antigens may be required. This may help to prevent tumor escape via down-regulation of any single target antigen. Data concerning basic immunological mechanisms and antigen presentation in the CNS that are gained through basic studies should improve the chances of success with immunological antitumor strategies.

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