T cell activity in glioma chemoresponsiveness and genetics
Review Article

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Summary
The dismal prognoses suffered by malignant primary brain tumor (glioma) patients remain unchanged over the past two decades despite significant improvements in the treatment of distinct tumors. Immunotherapy, and vaccine therapy in particular, represents a promising experimental approach to treat malignant gliomas, but major challenges still remain to render vaccination clinically effective. Combining vaccination with distinct therapies may be beneficial in this regard. For example, clinical chemotherapeutic responsiveness of glioblastoma multiforme (GBM) appears enhanced after therapeutic vaccination, and correlates with age-associated levels of nascent CD8⁺ T cells in patients. This review places these findings in the backdrop of previous research into glioma immunity, and introduce preliminary data validating a causative influence of T cells on glioma chemosensitivity. Further preliminary data suggesting an increase in a chromosomal marker of chemosensitivity in glioma tissue after therapeutic vaccination, and its bearing on the mechanism of T cell-induced glioma chemosensitivity is also discussed. Further insight into the dynamics of immune-tumor interactions promises to extend the reach of vaccine therapy by delineating the potential for immune synergy with conventional or targeted treatments.

I. Introduction
Malignant brain tumors are among the gravest forms of cancer. The most common of these incurable tumors, glioblastoma multiforme (GBM), is responsible for 50% of all intracranial gliomas and 25% of intracranial tumors in adults (Davis et al, 2001; DeAngelis, 2001). GBM diagnosis carries with it an average survival between 12 and 18 months (with 90-95% patients surviving less than 2 years), without the possibility of spontaneous remission or effective treatment (Kaplan, 1993; Davis et al, 2001; DeAngelis, 2001). The consistently short survival and absence of spontaneous remission that makes GBM such a devastating disease also renders the evaluation of new therapies for GBM relatively rapid and unequivocal. Overall survival represents the standard by which therapies for GBM are evaluated, in part because tumor mass reduction (i.e., surgically) does not necessarily correlate with prolonged survival (Kreth et al, 1993; Quigley et al, 1995; Hentschel and Lang, 2003).

A. Treatment for malignant gliomas
Unfortunately, conventional therapies fail to substantially improve GBM clinical outcome despite their ability to confer significant benefits to patients with non-glioma tumors (Kaplan, 1993; Reavey-Cantwell et al, 2001; Stupp and Hegi, 2003). Surgical resection followed by radiation and chemotherapy remains the most effective treatment (Kreth et al, 1993; Lacroix et al, 2001), but surgical resection options are limited by the involvement of vital brain structures. Moreover, the clinical impact of surgery followed by radiation on GBM is manifested as a small increase in survival that is evident primarily in large population studies (Kreth et al, 1993; Lacroix et al, 2001). Chemotherapy minimally improves GBM outcome, and
does so primarily in young patients (Fine et al, 1993; Diete et al, 2001). These treatment failures stem, in part, from the fact that GBM cells are by nature highly invasive, such that even radical surgical resections leave disseminated, invasive tumor cells, and thus fail to influence recurrence (Dandy, 1928; Burger, 1983). In addition, the normal brain is indispensable, yet highly sensitive to cytotoxic treatments, limiting effective treatment dosages (Feun et al, 1984; Kapp and Sanford, 1986; Oldfield et al, 1987; Ichikawa et al, 2000). These factors create the need for more specific GBM treatment modalities.

Although gene therapy for GBM remains a hopeful approach in this regard, its clinical translation remains a goal for the indeterminate future (Van et al, 2004). In contrast, adaptive immune-based therapies for GBM carry the potential for exquisitely specific destruction of tumor cells, as well as long-term protection against recurrence (Ehtesham et al, 2004). Accordingly, initial therapeutic vaccine trials for GBM have proven safe, and consistently hopeful (Kikuchi et al, 2001; Yamanaka et al, 2003). Thus, the immediate improvement of vaccine therapy for GBM is justified. Clinical improvement of current GBM vaccines can perhaps best be realized by understanding how T cell activity may alter properties of GBM tumors in ways that sensitize them to additional therapies. With such understanding, immunotherapy can be optimally combined with other treatments to achieve tangible clinical responses.

B. Cancer vaccines and dendritic cells: historical overview

An immunological influence on tumor rejection has long been recognized. Even before the advent of inbred strains of mice, it was discovered that transplants of tumors originating in white mice would grow in other white mice, but were rejected when transplanted into nondescript wild mice (Jensen, 1903). This ultimately led to the concept of tumor antigens (Haldane, 1933) that can initiate immune responses resulting in the destruction of susceptible tumors (Gorer, 1938). Not until the late 20th century, however, was it demonstrated that immune effector cells (CD8+ cytotoxic T cells or CTL) could kill tumors by recognizing tumor antigens bound to MHC I molecules (Zinkernagel and Doherty, 1979; Van and Boon 1982; van Bruggen et al 1991).

Tumor immunotherapy, and indeed any immune response against tumors, requires the expression of a target antigen on neoantigen cells. The derivation of tumor antigens was long presumed to be from self molecules altered within neoantigen cells so as to appear “foreign” to the host immune system. It was somewhat surprising, then, that many antigens mediating the rejection of human tumors were found to be essentially unaltered self molecules involved in routine functions in the affected tissue (Boon et al, 1994; Rosenberg, 1997). This paradox was partially resolved by the realization that tumor cells themselves were not the exclusive in vivo presenters of MHC I-restricted antigen to immune cells, but rather this was a function of a specialized group of “professional” antigen-presenting cells, dendritic cells (DCs), that could process self antigens for presentation on MHC I (Inaba et al, 1990).

Therapeutic vaccination of cancer patients has enjoyed a surge in popularity as an experimental clinical platform with the demonstration that ex vivo-generated DCs can stimulate curative anti-tumor T cell responses to established tumors in experimental rodents (Inaba et al, 1990; Mayordomo et al, 1995; Zitvogel et al, 1996). In these model systems, T cell responsiveness coincided with treatment efficacy (Sampson et al, 1996; Zitvogel et al, 1996; Gong et al, 1997; Okada et al, 1998; Wang et al, 1998; Matsui et al, 1999; Walker et al, 2000). As comparable DC populations were identified in humans (Romani et al, 1994), the notion that similar DC vaccines could be used to treat cancer patients gained favor. Early DC vaccine clinical trials in lymphoma and melanoma were initiated that provided a backdrop for the adoption of dendritic cell (DC)-based vaccine therapies in a variety of human tumors (Hsu et al, 1996; Nestle et al, 1998), including prostate cancer (Murphy et al, 1999a, b; Fong et al, 2001), renal cell carcinoma (Holdt et al, 1999; Kugler et al, 2000), NSC lung carcinoma (Fong et al, 2001), colon cancer (Morse et al, 2000), and malignant glioma/GBM (Kikuchi et al, 2001; Yu et al, 2001, 2004; Yamanaka et al, 2003). As this form of vaccination was increasingly applied clinically, a majority of patients typically exhibited induction of anti-tumor T cell responses. Relatively few patients, however, experienced tangible clinical benefits, and such benefits were generally unrelated to T cell responsiveness (Panelli et al, 2000). This may be due to the ability of tumors to evade host immunity not only by actively suppressing immune induction and/or effector function, but also through the development of immune resistant tumor variants under immune selection pressure. A complete appreciation of such limitations requires a broader understanding of immune processes and cell types.

II. Therapeutic cancer vaccines aim to mobilize antigen-specific T cells

Of the two basic types of immune cells (B and T) capable of adaptive (i.e., memory) responses, only T cells respond predominantly to cell-derived antigen (Ag; usually short peptide segments). They do so by producing cytokines and/or killing their Ag-expressing target cells. As such, T cells are most relevant for destruction and long-term protection against tumors. Most vaccine strategies, and DC vaccination in particular, seek to mobilize tumor-specific T cell responses (Rosenberg, 1997).

A. Molecular and cellular interactions in T cell Ag responsiveness

Small subsets of T cells bear “gamma delta” Ag receptors (γδ T cell receptors, or TCRγδ), and are capable of responding directly to some types of self and/or stress-induced Ag, including those on tumors (Rock et al, 1994, Schild et al, 1994, Morita et al, 1995, Groh et al, 1999). Recent evidence also suggests that these γδ T cells may serve to bridge innate and adaptive immune responses by
serving as professional antigen-presenting cells themselves (Brandes et al, 2005). The vast majority of T cells, however, express “alpha beta” TCR (TCRαβ), and respond to Ag (including tumor Ag) through TCR binding to peptide Ag which is itself bound to MHC molecules (designated HLA in humans) on distinct cells (Figure 1). The TCRαβ is aided in this process by one of 2 coreceptors, CD4 or CD8, whose mutually exclusive expression defines the two most prevalent types of T cells. CD4+ and CD8+ T cells bind distinct types of Ag/MHC (Norment et al, 1988; Salter et al, 1990; Konig et al, 1992). The CD4 and CD8 glycoproteins themselves act as “coreceptors”, binding to non-peptide-binding portions of the same type of peptide/MHC that engages the TCR, and juxtaposing critical kinases (p56lck and LAT, for example) close to their TCR-associated signaling (CD3) moieties in the process (Letourneur et al, 1990; Abraham et al, 1991; Miceli et al, 1991). In this manner, TCR ligation, signaling, and T cell activation is facilitated (Figure 1).

CD8+ T cells recognize predominantly intracellular peptide Ag bound to ubiquitously expressed MHC class I (MHC I) molecules (HLA-A, B, C in humans), and give rise to cytotoxic T lymphocytes (CTL) that can directly kill Ag/MHC I-bearing cells such as tumors (Braciale et al, 1987; Sweetser et al, 1989). CD4+ T cells (helper T cells, or Th) recognize predominantly endocytosed peptide Ag bound to MHC class II (MHC II) molecules (HLA-DR, DQ, DP, DO in humans) expressed on some myeloid and lymphoid blood cells (Braciale et al, 1987; Adorini et al, 1990). Depending on environmental and/or intercellular signals, Th cells can differentiate into Th1 and Th2 subtypes (Seder and Paul, 1994). Th1 cells secrete a particular array of cytokines (eg., IL-2, IFN-γ, IL-12) that promote CTL responses. Thus, Th1 cells and/or CTL responses are most relevant for inducing and monitoring anti-tumor immunity (Surman et al, 2000).

Once activated, naïve T cells develop “memory” function and phenotypes, able to respond more quickly and efficiently to challenge with their activating antigen. Post-activation proliferative expansion of memory type T cells in vivo (i.e., homeostasis) can, however, proceed at least to an extent independently of antigenic stimulation. Such homeostatic expansion is controlled by cytokines including IL-2, IL-7, and IL-15, and regulatory T cells (Marrack et al, 2000, Murakami et al, 2002, Sprent & Surh, 2003, Judge et al, 2002, Kieper et al, 2002, Tan et al, 2002). Although such homeostatic expansion of memory T cells may be relevant to anti-tumor activity, it is more generally believed that novel activation of naïve rather than pre-existing memory T cells may mediate effective immunity in tumor hosts (Schweighoffer et al, 1996). Altered memory T cell homeostasis may, however, contribute to age-related deficits in T cell function that also appear in glioma patients (Ku et al, 2001, Clambey et al, 2005, Morford et al, 1997).

The importance of T cell activity in vaccine-mediated survival benefits is readily apparent in rodent tumor vaccine models, in which increased survival and protection are clearly dependent on the presence of CD4+ or CD8+ T cells (Dranoff et al, 1993; Sampson et al, 1996; Zitvogel et al, 1996; Gong et al, 1997; Okada et al, 1998; Wang et al, 1998). In many cases, both CD4+ and CD8+ T cells are essential to transfer therapeutic benefits to naïve hosts. In some intracranial tumor models, however, CD8+ T cells alone appear to mediate such benefits (Sampson et al, 1996; Walker et al, 2000). In nearly all rodent tumor

**Figure 1.** Molecular interactions in T cell signaling and activation. Class I or class II major histocompatibility complex (MHC) molecules bind and present peptide antigen (triangle on MHC) to T cells expressing either CD8 or CD4 coreceptors, respectively. CD4 and CD8 coreceptors can bind to the same MHC molecule as is bound by the T cell receptor (TCR), and help localize intracellular signaling proteins, such as lck or LAT, to transmembrane signaling proteins (CD3) associated with TCR, thereby potentiating a signaling cascade that ultimately leads to cellular activation.
vaccine models, increased memory CTL activity correlates with enhanced survival upon vaccination (Sampson et al, 1996; Zittvogel et al, 1996; Gong et al, 1997; Okada et al, 1998; Wang et al, 1998; Matsui et al, 1999; Walker et al, 2000). As well, the importance of CD8 expression itself in anti-glioma activity is underscored by its specific loss in defective glioma-infiltrating CTL (Prins et al, 2001, 2004).

III. Obstacles to effective anti-glioma immunity

A. Evidence of endogenous immune suppression

Tumors in general can compromise anti-tumor immunity, either at the level of T cell response induction or effector function. With respect to gliomas, pioneering work demonstrated that these tumors inhibit T cell response induction (Brooks et al, 1976, 1977, 1981; Mahaley Jr et al, 1977; Woosley et al, 1977; Menzies et al, 1980; Gately et al, 1982; Wood and Morantz, 1983; Elliott et al, 1984; Ausiello et al, 1988, 1991; McVicar et al, 1992; Morford et al, 1997). Suppressive cytokines such as TGF-β, IGF-1, prostaglandin E2, and IL-6 were eventually implicated, largely from in vitro studies, in the inability to induce anti-tumor T cell responses (Bodmer et al, 1989; Black et al, 1992). The release of these cytokines, as well as other less defined factors (Bodmer et al, 1989; Kuppern et al, 1989; Black et al, 1992; Lafarge-Frayssinet et al, 1997; Parney et al, 2000), has also been postulated to be a response by the tumor to immune infiltration (Black et al, 1992). These potential impediments to glioma anti-tumor immunity serve to “cloak” the tumor from T cell responsiveness at the level of immune induction. These initial findings fueled suspicion that strong endogenous anti-tumor immune responses were neither possible nor relevant to clinical outcome in glioma patients (Weller and Fontana, 1995).

It was later shown that T cells from high-grade glioma patients exhibited intrinsic defects in an array of signaling molecules similar to those seen in other cancer patients (Matsuda et al, 1995; Lai et al, 1996; Rabinovich et al, 1996; Morford et al, 1997; Reichert et al, 1998). Importantly, the severity of these T cell defects was correlated with glioma size, consistent with notions that a glioma-derived factor elicited the defects (Morford et al, 1997), and/or that dysfunctional immune effectors exacerbated glioma progression. Although a tumor-derived factor responsible for T cell defectiveness in glioma patients has not yet been definitively identified, alternative mechanisms generating T cell defects that also exacerbate tumor progression have now been validated in glioma-bearing mice (Prins et al, 2004). This suggests that host T cell competence could have some bearing on clinical outcome in glioma patients.

B. Immune induction in glioma vaccine trials

The evidence that endogenous cellular immune suppression might worsen glioma progression does not necessarily mean that bolstering cellular immunity through vaccination can improve clinical outcomes in glioma patients. In addition, the ability of DCs or any other means to activate anti-tumor T cells in immune-suppressed glioma patients is by no means a foregone conclusion, regardless of the relevance of analogous endogenous immune processes. It is necessary to demonstrate induction of anti-tumor T cell responses and to monitor associated clinical outcomes in GBM patients in this regard. These have been explicit goals of therapeutic DC vaccine trials.

Results from four DC glioma vaccine trials have now been published, and the details of which are reviewed elsewhere (Wheeler and Black, 2005). Briefly, these trials demonstrate that T cell responses can indeed be induced in high-grade glioma patients, despite concerns over T cell suppression. To date, however, only one of these trials provided recognizably fallible evidence that is nonetheless consistent with improved clinical outcome after vaccination in GBM patients (Yu et al, 2004).

IV. Improving glioma vaccines

Identifying the cells capable of slowing or halting tumor progression in cancer patients, and/or identifying the critical effector functions of the immune system in counteracting human tumor progression, are required to improve clinical cancer vaccines. While such a cellular basis of beneficial immunity is readily apparent in experimental animals, upon successful transfer of protective immunity by CD8+ and/or CD4+ T cells, the failure of a variety of T cell indices to correlate with clinical benefits in vaccine trials makes this a much more daunting task in patients (Panelli et al, 2000). Similarly, administration of DC vaccines to cancer patients has failed to elicit the relatively dramatic affects expected of this therapy based on early animal studies, revealing that antigen-pulsed DC administration is not likely to constitute the sole limitation to clinically effective anti-tumor immunity in patients, as it so often does in experimental rodents (Bodey et al, 2000). Early evidence documenting specific enrichment of newly-produced CD8+ T cells (recent thymic emigrants, or RTEs) that are rare in adult humans but prevalent in experimental rodents, within tumor-infiltrating T cells in glioma-bearing rats (Okada et al, 2003), suggested a cellular candidate mediating additional immune limitation. Peripheral levels of CD8+ RTEs also uniquely correlate with levels of CD8+ T cells infiltrating human GBM (r = 0.92; P < 0.03) whereas total peripheral CD8+ T cells do not (r = 0.15; P > 0.7), suggesting that CD8+ RTEs are similarly relevant in GBM patients (CJ Wheeler, KL Black, unpublished data), and prompting for further analysis of GBM patients.

A. CD8+ RTE activity and dominance

Tumor antigen reactivity, pre-existing RTE levels and post-vaccine responses were quantified in glioma patients, and tested for the ability to predict clinical outcomes in GBM patients. Using molecular as well as phenotypic markers for RTEs (Douek et al, 1998; McFarland et al, 2000), the presence and post-vaccine responsiveness of CD8+ RTEs were found to not only accurately predict age-dependent clinical outcome in GBM patients in general, but to largely account for the
influence of age, the strongest established prognostic factor (Figure 2) (Wheeler et al., 2003). In addition, glioma-bearing mice specifically deficient in thymic production of CD8+ RTEs, but not peripheral CD8+ T cell activity, also exhibited decreased age-dependent survival and strong correlation between thymic cell production and clinical outcome as was observed in human GBM patients (Figure 3). This suggests that CD8+ RTE production may critically influence age-dependent glioma host survival in human patients and in mice deficient in their generation, but not in wild-type mice typically used for experimental tumor studies (Wheeler et al., 2003). The level of CD8+ RTE proliferation and/or migration in patients’ peripheral blood also correlated strongly (r = 0.96) with type I cytokine response in vitro in DC-vaccinated GBM patients (Wheeler et al., 2003). Moreover, the vast majority of T cells binding any of 4 distinct tumor Ag/HLA multimers exhibited a CD8+ RTE phenotype, with related activated cells specifically expanded in vivo upon vaccination. Finally, calculated numbers of CD8+ RTEs responding in vivo uniquely predicted both disease-free and overall GBM patient survival following DC vaccination. Taken together, these findings suggest that (i) the CD8+ RTE subpopulation may possess intrinsically high tumor reactivity, (ii) that levels of these cells are limited prior to vaccination in many GBM patients, and (iii) the number of these cells responding in vivo determines the degree to which they account for clinical outcome in vaccinated GBM patients. Thus, these cells appear to directly mediate and critically limit beneficial anti-glioma immune responses, and dominate over other responding T cells in this regard upon DC vaccination. This emerging knowledge affords extraordinary opportunities to rationally improve existing DC-based therapies, as well as to probe immune influences on established glioma characteristics in patients.

Figure 2. TREC within purified T cells, a molecular parameter quantifying RTE levels, account for age-dependent GBM outcome. Patients were separated for analyses by the indicated parameters above or below their median values in the entire population, in CD8 TREC-matched cohorts, or in age-matched cohorts as indicated, and Kaplan-Meier analysis was performed. Open circles reflect censured clinical outcome data. Each cohort patient was matched for either age (36-66 yrs range in each cohort; n = 10/cohort; P = 0.96) or CD8+ TREC (1.5-4309.5 and 0.6-5530.4 ranges in old and young cohorts, respectively; n = 11/cohort; P = 0.86), to a counterpart with distinct CD8+ TREC (P < 0.05) or age (P < 0.008), respectively. Expansion to right depicts contribution of non-vaccinated patient subgroup to ability of CD8 TREC to predict survival. 2-tailed Mann-Whitney log-rank tests for disease-free and overall survival were calculated with SAS software. Reproduced from Wheeler et al., 2003 with kind permission from Journal of Immunology.
B. Immunoediting and glioma immune susceptibility

Active suppression of immunity in tumor hosts may afford the tumor a critical growth advantage under a wide variety of conditions. From one perspective, the goal of immunotherapy is to break free from or overwhelm such suppressive mechanisms to substantially destroy tumor cells in situ. Tumors such as malignant gliomas are highly genetically plastic, however, and as such may be able to evade immune destruction by altering expression of their own intrinsic immune susceptibility genes. This mode of immune evasion was first realized clinically when vaccinated lymphoma patients experiencing long post-vaccine remissions, suffered recurrence by tumors devoid of immunizing antigen, of antigen processing machinery, or of appropriate HLA restriction elements (Jager et al., 1996, 1997; Ohmacht et al., 2001). Later, it was also shown that the immune effector cells and molecules collaborate to stably alter tumor malignant behavior when subjected to immune influence in rodents (Shankaran et al., 2001; Dunn et al., 2004). Such evasiveness is distinguished from active immune suppression in that it does not impair the inherent ability of immune cells to carry out their effector functions. This distinction is important, because focusing on active immune suppression encourages strategies to enhance immune function, whereas focusing on tumor immune evasion encourages a very different strategy of bypassing or exploiting the tumor’s ability to adapt to immune selective pressure. Conceivably, immune enhancement could even speed the development of immune-resistant tumor variants, a possibility that could worsen clinical outcomes. In this regard, documenting and understanding the development of resistance to immune-mediated destruction of tumors may be a key to successful immunotherapy for cancer.

Immune-tumor interaction concepts have recently been overhauled, and a discussion of these changes helps contextualize recent work in vaccinated glioma patients pursuant to monitoring and understanding the development of immune resistance in tumors. The immunoediting model put forth by Schreiber and colleagues (Dunn et al., 2002) updates the earlier concept of immune surveillance, wherein host immunity prevents the development of nascent tumors, and thereafter becomes irrelevant (Burnet, 1970). This new model, substantiated by a growing body of experimental evidence (Smyth et al., 2001; Dunn et al., 2002, 2004), holds that tumors undergo 3 distinct phases of interaction with host immunity: elimination, equilibrium, and escape (Figure 4). Elimination refers to the complete immune-mediated destruction of nascent tumor cells prior to their establishment, essentially embodying the original immune surveillance hypothesis (Dunn et al., 2004; Burnet, 1970). Equilibrium refers to a further latent phase of tumor establishment wherein immune activity effectively kills off the most immune-sensitive tumor cells, while failing to similarly eradicate less susceptible tumor subpopulations. This dynamic leads to the eventual selection of tumors that are immune resistant, whose growth outstrips immune constraints in the escape phase. The strong influence of CD8+ RTEs on GBM clinical outcome suggests that these tumors may generally exist in a transitory phase between equilibrium and escape. Thus, hope that continued improvement in bolstering anti-glioma immunity will result in ever-increased slowing of glioma progression is tempered by the likelihood that such slowing will quickly reach an asymptotic limit due to the selection of immune-resistant tumor variants.

The concept that glioma progression may be slowed below a finite level of T cell immunity, yet potentially exacerbated by hastening the development of immune
resistant tumors above that level is consistent with our recent findings. GBM patient groups that, on average, experience lower levels of immune response enhancement following vaccination also appear to enjoy significantly prolonged survival, whereas patients exhibiting greater average immune responses after vaccination fail to exhibit prolonged survival (CJ Wheeler, KL Black, unpublished data). Resolution of this conundrum can only come from understanding how immune selective forces fundamentally alter gliomas. Based on such understanding, the restoration of immune susceptibility by reversing immune-induced changes could be attempted. Alternatively, an attempt could be made to determine whether CTL responses that do not result in net tumor destruction nevertheless constrain glioma cells in ways that are therapeutically exploitable.

C. Bypassing immune limitations in glioma patients: post-vaccine chemosensitization

A critical property of clinically effective anti-tumor immune effector cells is the ability to reproducibly alter large proportions of tumor cells in situ. Ideally, this would involve the wholesale destruction of tumor cells, but net tumor growth may also be constrained in less obvious ways. For example, recent evidence suggests that GBM tumors recurring after vaccination may be more sensitive to conventional chemotherapy than recurrent tumors in non-vaccinated patients (Wheeler et al, 2004).

Figure 4. The Immunoediting model in the context of glioma-immune dynamics. Tumor cells susceptible to destruction (blue) by immune cells (activated CD8⁺ RTE progeny; green) initially grow interspersed with normal (gray) cells in the brain, leading to antigen uptake by nearby antigen-presenting cells (yellow-orange) and distal activation of CD8⁺ RTEs. Activated progeny of CD8⁺ RTEs then localize to the tumor site and eradicate most tumor cells in the elimination phase. To the extent that this process is not absolutely successful, tumor cells may enter the equilibrium phase, wherein immune-resistant tumor cells are selected to produce a new population of tumor variants in the equilibrium phase. The elimination, and possibly the equilibrium phase likely precede clinical tumor presentation. Immune-resistant tumor cells whose growth rate exceeds that of immune-mediated tumor cells destruction become clinically detectable in the escape phase. These immune-selected tumor cells are proposed to be rare or absent in the initial glioma cell population, uniquely chemosensitive, and possessing a growth advantage over other tumor variants specifically under conditions of strong anti-tumor immunity. Chemo-resistant tumor variants (orange), with unknown susceptibility to immune attack, would be similarly selected following inefficient chemotherapeutic tumor destruction. Reproduced from Dunn et al, 2002 with kind permission from Nature Immunology.
Although originating from distinct clinical studies not designed to address synergy between vaccination and chemotherapy, empirical validation allowed a comparison among three patient groups treated with either vaccine or chemotherapy alone, or with chemotherapy after vaccination (Wheeler et al, 2004). Vaccinated patients receiving subsequent chemotherapy exhibited significantly delayed tumor progression and longer survival relative to those receiving vaccinations without subsequent chemotherapy or to those receiving chemotherapy alone (Figure 5). Multiple patients also exhibited objective

![Figure 5](image)
(>50%) regression of tumor burden, an extremely rare phenomenon in GBM (Figure 5). Improved clinical outcome appeared dependent on the specific combination of therapeutic vaccination followed by chemotherapy, suggesting a substantial therapeutic slowing of GBM progression and extension of overall patient survival that appeared to markedly surpass that in previous vaccine as well as chemotherapy studies in high-grade glioma patients (Stupp and Hegi, 2003). Although both glioma clinical outcome and chemotherapeutic responsiveness are age-dependent, a stronger correlation existed between CD8+ RTEs and chemotherapeutic responsiveness than between age and chemotherapeutic responsiveness, and CD8+ RTE levels predicted a significant increase in such responsiveness (Figure 6).

This study suggests that T cell immune activity, mediated predominantly by CD8+ RTEs, appears insufficient to eradicate gliomas in situ, but also confers enhanced sensitivity of the glioma to genotoxic agents (i.e., various forms of chemotherapy). An additional study describes GBM regression following post-vaccine chemotherapy. Evidence consistent with tumor recurrence after vaccination in this study, however, was interpreted as inflammatory response, leading to the conclusion that subsequent tumor regression after chemotherapy was elicited by vaccination alone (Okada et al, 2003). We suspect that GBM regression in this study, which utilizes IL-4-expressing glioma cells rather than antigen-pulsed DCs as vaccine, is also due to post-vaccine chemotherapy rather than vaccination alone. This alternate interpretation is more consistent with the notion that immune-selected GBM cells, regardless of the means of initiating immune selection, are particularly chemo-sensitive. We propose that the dominant cellular mediators of such selection are CD8+ RTEs, the affects of which on glioma composition can be easily visualized in the context of the immunoediting model (Figure 4).

1. In vivo model of T cell-induced glioma chemosensitivity
To address whether increased T cell activity is able to directly select chemosensitive tumors, it is first necessary to demonstrate causality in an animal glioma model. To this end, GL26 glioma cells were implanted intracranially into T cell-deficient (nude) or syngeneic wild-type (C57Bl/6) mice, which were treated with chemotherapeutic drugs alone or with vaccination using GL26 lysate-pulsed DC2.4 dendritic cells. Treatment with either minoxidil sulfate or temozolamide elicited distinct results in nude mice, with minoxidil sulfate exhibiting no survival increase and temozolamide eliciting a small increase (5 day mean survival increase; Figure 7). In contrast, both drugs enhanced survival in wild-type hosts to a greater, with minoxidil sulfate eliciting a survival increase with or without vaccine treatment, and temozolamide lengthening survival in vaccine-treated hosts (10 day mean survival increase; Figure 7). Vaccine treatment of wild-type mice enhanced anti-GL26 CTL responsiveness and increased survival somewhat, but this effect was not significant by itself (P > 0.05; not shown). Thus, T cell activity directly enhanced in vivo glioma drug responsiveness.

Figure 6. Levels of nascent CD8+ T cells (CD8+ recent thymic emigrants, or CD8+ RTEs) are strongly associated with chemotherapeutic responses following vaccination. TREC, a molecular measure of RTEs, within purified CD8+ T cells collected at the time of surgery were correlated with the increase in time to tumor progression (time to recurrence after chemotherapy minus time to recurrence after vaccination in the same patient; Top). Patients were subdivided based on median CD8+ TREC level, and Kaplan-Meier survival analyses conducted (Bottom). Data were derived from all vaccinated de novo and secondary GBM patients for whom chemotherapeutic response and TREC results were available (n = 12). Correlations with and predictive power of patient age or IFN-γ response magnitude were not statistically significant. Reproduced from Wheeler et al, 2004 with kind permission from Clinical Cancer Research.
2. Genetics of T cell-induced glioma chemosensitivity

Because chemosensitivity of gliomas, including GBM, has been linked to tumor genetics (Leuraud et al., 2004), considering these data in the context of immunoediting suggests that immune selection may drive in situ glioma evolution away from a chemo-resistant genotype, and toward a chemosensitive one (Figure 4). Demonstration of relevant genetic change in post-vaccine GBM tissue would support the notion that immune activity selects glioma cells with greater chemosensitivity. We therefore examined loss of heterozygosity (LOH) near 1p36 by differential hybridization to polymorphic microsatellite markers, since both of these events (particularly 1p36 LOH) are associated with chemotherapeutic responsiveness in astrocytic and oligodendrocytic gliomas (Cairncross et al., 1998; Fortin et al., 1999; Smith et al., 1999; Bauman et al., 2000; Ino et al., 2000). Because large chromosomal tracts are disjointed in mice relative to humans and 1p36 LOH is not a defined phenomenon in mouse gliomas, we confined our analysis to human GBM tissue. We first identified demographic groups of de novo GBM patients that were responsive or non-responsive to post-vaccine chemotherapy, and then assessed LOH frequency in pre- and post-vaccine tumor tissue from these patients. Pre- and post-vaccine GBM tissue from the same patients revealed increases in 1p36 LOH specifically in responders after vaccination. Most of the increase of 1p36 LOH frequency in responders was accounted for by the 1p36.1 marker, D1s214 (Figure 8). Control GBM tissue isolated from non-vaccinated GBM patients of the same age and at analogous intervals did not exhibit any 1p36 LOH initially, and this did not change in subsequent samples (not shown). Moreover, of 12 loci screened for LOH using 42 individual microsatellite markers, 1p36 LOH represented the single most striking post-vaccine alteration in chemotherapeutically responsive patients (not shown). Thus, a chromosomal marker of glioma chemosensitivity was specifically increased after vaccination, and this occurred preferentially in chemotherapeutically responsive GBM patients. This directly supports the notion that T cell activity selects chemosensitive glioma variants. A valid alternative notion explaining the glioma outcome data, that post-vaccine chemotherapy only enhances anti-tumor immune responses by selectively killing suppressor T cells, is inconsistent with this genetic data.

V. Conclusion

The field of cancer vaccination has witnessed substantial progress in the past 5 years. Clinical cancer vaccines in general, including those for GBM and other high-grade gliomas, have progressed to the point that they consistently elicit tumor-specific CTL expansion in a majority of recipients (Kikuchi et al., 2001; Yu et al., 2001, 2004; Yamanaka et al., 2003). Impressive clinical responses have been observed, but in general these still occur in small subgroups of patients (Nestle et al., 1998; Kugler et al., 2000; Fong et al., 2001; Wheeler et al., 2004). In addition, unlike in rodent tumor vaccine models, clinical improvement in vaccinated cancer patients does not generally coincide well with anti-tumor memory T cell responses (Panelli et al., 2000). These observations suggest that vaccination is sufficient to elicit substantial tumor-destructive T cells in rodents, but that additional factors limit their tumor-destructive activity in vaccinated human patients (Bodey et al., 2000). In the past 5 years, glioma research has not only culminated in the successful launching of multiple clinical vaccine trials, but has also contributed significant milestones toward the goals of identifying and overcoming such obstacles to more effective therapeutic cancer vaccines.
Figure 8. 1p36 Loss of heterozygosity in post-vaccine GBM tissue. Tumor DNA from laser-dissected pre- and post-vaccine GBM specimens separated by vaccine-induced response demographics were analyzed for loss of heterozygosity (LOH) at 12 chromosomal loci previously implicated in glioma progression as previously described using polymorphic microsatellite markers (Newsham et al, 2000; Bogler et al, 2001). Each locus was monitored using at least 3 (1p36, 3p24-26, 9p21-24, 11p15, 13q14, and 17p13) or 4 (1p13-31, 10q22-26, 12q13-15, 18p11, 19q13, and 22q11-13) polymorphic markers per locus. Frequency of LOH at markers Ds12734 (1p36.1), D1s199 (1p36.1-21), Ds1214 (1p36.21-31) is shown in 6 responder and 6 non-responder patients. LOH frequency was defined by the number of patients exhibiting LOH (differential hybridization < 0.7 of tumor relative to autologous blood DNA in one of paired polymorphic markers) at informative markers divided by the total number of informative patients in the population. Combined 1p36 exhibited the greatest degree of post-vaccine LOH change among 12 tested loci (33% to 100%; n = 6 responders).

A discrete group of T cells involved in beneficial anti-glioma immunity has now been identified. This has allowed greater focus on the induction of T cell responses relevant to clinical outcome in the monitoring of DC vaccine trials for GBM patients, and thereby promises to link immunological with clinical endpoints in vaccine trials. In addition, this identification has revealed evidence that a specific subgroup of T cells (CD8^+^ RTEs) is unusually responsive to tumor antigens in general. Clearly, the further development of animal models that accurately reflect human glioma-CD8^+^ RTE interaction dynamics is necessary to address potential therapeutic applications of CD8^+^ RTEs in the context of adaptive or adoptive immunotherapy. Such models should also allow definitive examination of the potential impact of CD8^+^ RTEs on other forms of cancer as well. In addition, elucidating molecular and cellular mechanisms for the apparent dominance of human CD8^+^ RTEs in anti-glioma immunity, as well as salient effector mechanisms afforded by the activated progeny of these cells, may facilitate the enhancement of anti-tumor reactivity in less rare or otherwise suppressed T cells. Such efforts may also lead to improved clinical efficacy in glioma therapy.

No single treatment modality is likely to effectively diminish gliomas over long periods. In addition, several independent factors likely collaborate to encourage glioma progression. Clearly, the search for new immune and non-immune molecular targets for this disease must continue apace. It is also attractive in this regard to combine complementary therapeutic modalities in the quest for increasingly effective glioma therapies. In this regard, our preliminary studies suggest that immunotherapy may optimally complement subsequent chemotherapy to confer therapeutic benefits to glioma patients.

In the past year, evidence has been presented that vaccination, while ineffective alone in de novo GBM patients, may afford increased tumor sensitization to chemotherapy. This is particularly significant for GBM patients, in whom novel regressions of large tumor masses are now observed following post-vaccine chemotherapy. Definitive substantiation of post-vaccine glioma chemosensitization awaits the development of suitable animal models. In addition, the apparent efficacy of combining DC vaccination and chemotherapy, which is linked to tumor genetics in glioma, justifies further examination of how human glioma genotypes may be globally altered by anti-tumor immunity. This kind of genetic analysis may allow the identification of discrete genes/proteins mediating post-vaccine chemosensitivitiy in gliomas, as well as provide useful surrogates for the realization of post-vaccine chemosensitization in the clinic. Additionally, analysis of global vaccine-induced alteration of gliomas may provide further insight as to how gliomas evade immunity, and how such evasion may be successfully exploited therapeutically.

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