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DENDRITIC CELL VACCINES AND IMMUNITY IN GLIOMA PATIENTS

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1. ABSTRACT

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. These challenges include diminishing the risk of pathologic autoimmunity, and identifying the cellular basis of clinical vaccine benefits. Addressing such challenges should eventually help increase the proportion of patients experiencing clinical vaccine benefits. Recent studies in glioma patients have characterized tumor antigens on human gliomas, identified some of the immune cells involved in beneficial anti-glioma immunity, and examined how gliomas may be altered by sub-lethal immune influences. This has provided a glimpse of the strength to which immunity influences glioma clinical outcome, and resurrects hope that clinically effective vaccines to treat these tumors is within reach. Insight into the complex dynamics of immune-tumor interactions promises to extend this reach by delineating mechanisms of immune synergy with other forms of treatment.

2. 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 (1,2). 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 (1-3). 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 (4-6).

2.1. Treatment for malignant gliomas

Unfortunately, conventional therapies are remarkably ineffective at improving GBM clinical outcome despite their ability to efficiently treat patients with non-glioma tumors (3,7,8). Even the few treatments effective against GBM typically either exhibit small increases in survival that are evident only from large population studies,

or primarily benefit certain (i.e., young) patient subpopulations (9,10). Thus, novel therapies that overcome the failings of current GBM treatments are needed.

The reasons underlying the failure of conventional therapies for glioma may stem from their inability to meet several critical requirements. Prime among these requirements are that treatment must reach the entire volume of the CNS (glioma is a diffuse rather than local disease), should not be toxic to normal brain cells and structures (normal brain is indispensable), should limit the development of resistance to the therapy and should activate tumor killing if and when there is a recurrence. Analysis of these considerations is necessary to assess the realistic potential for success of any novel glioma therapy.

Cancer vaccines represent one novel therapy for GBM that fulfill the above requirements. Activated immune cells can survey the entire CNS with virtually unlimited access since there exists one capillary for every 2 neurons. In addition, activated immune (T) cells can cross the blood-brain-barrier (BBB). T cell killing of target cells, including tumors, can be exquisitely specific and need not be toxic to normal brain. Moreover, T cells retain memory for target (tumor) killing and should reactivate tumor killing if and when recurrence occurs. (11-13). The clinical efficacy of therapeutic vaccination for any human tumor, however, remains controversial because consistent tumor destruction or extended lifespan is not observed in most vaccinated cancer patients (14-16). In contrast, current cancer vaccines do reliably elicit tumor-reactive cytotoxic T lymphocytes (CTL) in most patients (14,15,17). The improvement of vaccine therapy for GBM and other cancer patients is contingent upon identifying and overcoming the mechanisms underlying their general clinical failure despite their experimental and apparent immunological success.

2.2. Cancer vaccines & 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 (18). This ultimately led to the concept of tumor antigens (19) that can initiate immune responses that lead to the destruction of susceptible tumors (20). Not until the late 20^{th} 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 (21-23).

Tumor immunotherapy, and indeed any immune response against tumors, requires the expression of a target antigen on neoplastic cells. The derivation of tumor antigens was long presumed to be from self molecules altered within neoplastic 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 functioning of the affected tissue (24,25). 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 that 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 (26).

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 (26-28). In these model systems, T cell responsiveness coincided with treatment efficacy (28-34). As comparable DC populations were identified in humans (35), 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 (36,37), including prostate cancer (38-40), renal cell carcinoma (41,42), NSC lung carcinoma (16), colon cancer (43), and malignant glioma/GBM (13,44-46). As this form of vaccination was increasingly applied clinically, a majority of patients typically exhibited induction of anti-tumor T cell responses. In contrast, relatively few patients experienced tangible clinical benefits, and such benefits were generally unrelated to T cell responsiveness (47). This may be due to the ability of tumors to evade host immunity not solely 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 general knowledge of immune processes and cell types.

3. 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 peptides). 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 (25).

3.1. Molecular & cellular interactions in T cell Ag responsiveness

T cells respond to Ag (including tumor Ag) through ligation of their Ag receptors (TCRs) by binding to peptide Ag which is itself bound to MHC molecules (designated HLA in humans) on distinct cells (figure 1). The TCR 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 (48-50). The CD4 and CD8 glycoproteins themselves act as "coreceptors", binding to non-peptide-binding portions of the same type of peptide/MHC that engages TCR, and juxtaposing critical kinases ($p56^{lck}$ and LAT, for example) close to their TCR-

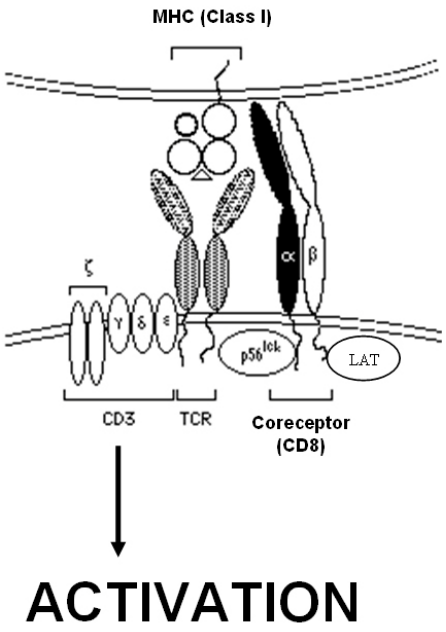

Figure 1. Molecular interactions in T cell signaling and activation. Class I or class II major histocompatibility complex (MHC) molecules bind and present peptide antigen (triangle on MHC) to T cells expressing either CD8 or CD4 coreceptors, respectively. CD4 and CD8 coreceptors can bind to the same 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.

ssociated signaling (CD3) moieties in the process (51-53). 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 (54,55). $CD4^+$ 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 (55,56). Depending on environmental and/or intercellular signals, Th can differentiate into Th1 and Th2 subtypes (57). Th1 cells secrete a particular array of cytokines (eg., IL-2, IFN-γ, IL-12) that promote CTL responses. Thus, Th1 and/or CTL responses are most relevant for inducing and monitoring anti-tumor immunity (58).

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 (28-32,59). 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 (29,34). In nearly all rodent tumor vaccine models, increased memory CTL activity correlates with enhanced survival upon vaccination (28-34). The importance of CD8 expression itself in anti-glioma activity is underscored by its specific loss in defective glioma-infiltrating CTL as well (60,61).

3.2. Activation of naïve anti-tumor T cells by dendritic cells

Although most T cell responses seen in tumor patients are recall "memory" responses, these can be inefficient and undermined through active suppression and/or loss of appropriately presented tumor Ag (62-64). Supression of previously activated T cells is particularly pronounced in gliomas (see below)(65-67). As a result, tumor vaccine efficacy may be dependent on "naïve" T cell responses to previously unrecognized tumor Ag (68). Such Ag can induce the activation of naïve T cells, provided it is presented by DCs, the most potent activators of naïve T cell responses (26,69). DCs' ability to prime naïve T cells is in part due to their expression of additional costimulatory molecules (B7, for example) that bind ligands (CD28) on naïve T cells, providing the additional signals necessary for the naïve T cell to proliferate and acquire effector function in response to tumor Ag (figure 2)(70). DCs are also unique in their ability to take up Ag endocytically from tumor cells and present it onto their own MHC I molecules (as opposed to MHC II; figure 2A)(71-73). DC activity thus represents a means to initiate novel T cell killing responses against otherwise non-immunogenic tumor cells.

Normally, DCs are present in very small numbers in the circulation. For this reason, and because naïve T cells reactive to specific Ag may be even more scarce, endogenous anti-tumor immunity may be limited by the rate of encounter between these two cell types. Experimental vaccines that bypass this limitation by administering large numbers of tumor Ag-pulsed, *ex vivo*-generated DCs to hosts have been extremely successful against many experimental tumors in rodents, including intracranial gliomas (12,27,28,74-76). Even this approach is expected to fail, however, if tumor Ag-specific T cells fall below a certain frequency (see below, figure 6).

When a specific naïve ($CD8^+$) T cell first encounters Ag (usually on DCs in lymphoid tissue such as lymph nodes or spleen; figure 2B), it becomes activated to proliferate, and differentiates to acquire effector function (figure 2C). In this process, the T cells up-regulate proteins that allow them home to, enter into and travel through non-lymphoid tissues, including brain (figure 3)(77,78). The blood brain barrier does not prevent the entry of these metabolically active cells (79), that are triggered to carry out their effector functions (cytokine production, killing) once they re-encounter their antigen within tumor sites (figures 2C, 3).

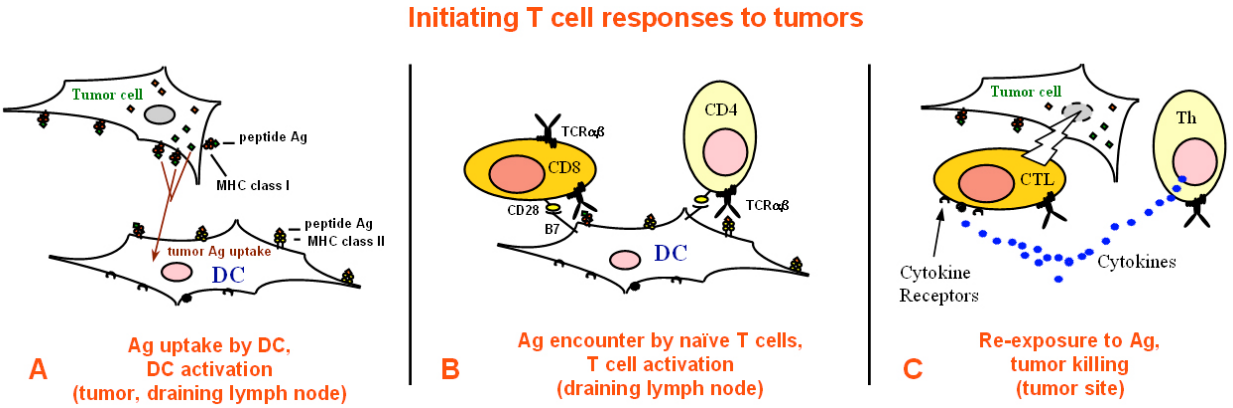

Figure 2. Initiating T cell responses to tumors. Dendritic cells (DCs), resident either within the tumor or in draining lymph nodes, take up tumor antigen, fragment it into peptides, and present these peptides on their MHC molecules (A). $CD8^+$ or $CD4^+$ T cells with receptors (TCRαβ) able to bind these peptide/MHC complexes encounter DCs in draining lymph nodes, where they are activated to become cytotoxic T lymphocytes (CTL) or T helper cells (Th), respectively (B). CTL and Th recounter cognate antigen at the tumor site, and collaborate to elicit optimal anti-tumor effector activity.

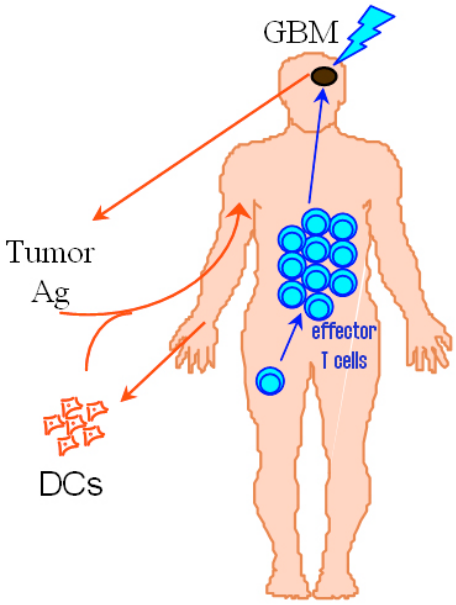

Figure 3. Vaccine-elicited effector T cell response. Dendritic cell-based (DC) vaccines generate large numbers of tumor antigen-presenting DCs *ex vivo*, bypassing a potential limitation to endogenous immune activation in patients. Re-administration of tumor antigen-pulsed DCs as vaccine aims to elicit expansion and anti-tumor effector functions in tumor-reactive T cells, which can then migrate to and kill tumors *in situ*.

4. OBSTACLES TO EFFECTIVE ANTI-GLIOMA IMMUNITY

4.1. 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 (65,66,80-90). Suppressive cytokines such as TGF-β, IGF-1, prostaglandin E_2, and IL-6 were eventually implicated, largely from *in vitro* studies, in the inability to induce anti-tumor T cell responses (91,92). The release of these cytokines, as well as other less defined factors (91,93-95), has also been postulated to be a response by the tumor to immune infiltration (92). 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 (96).

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 (66,97-100). Importantly, the severity of these T cell defects was correlated with glioma size, consistent with notions that a glioma-derived factor elicited the defects (66), 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 (61). This suggests that host T cell competence could have some bearing on clinical outcome in glioma patients.

At the level of immune effector cell survival, FasL expression on glioma vasculature, which could lead to infiltrating T cell apoptosis, has been correlated with the preponderance of $CD4^+$ helper over $CD8^+$ killer T cells infiltrating patients' gliomas (101). This is consistent with the differential susceptibility to fas-mediated cell death among $CD4^+$ and $CD8^+$ T cells, as well as a skewing of local anti-glioma immunity away from CTL promotion. In addition, thymic production of nascent $CD8^+$ T cells (recent thymic emigrants, or RTEs), which may be particularly important in countering glioma progression (102), is

dramatically diminished due to intrinsic apoptosis as a result of glioma growth in rats (103). Finally, non-neoplastic normal astrocytes themselves have been reported to suppress both T cell activation and effector function through upregulation of CTLA-4, a negative regulatory molecule that binds competitively to CD28 on T cells (104). This illustrates a mechanism that potentially contributes to the generally refractory nature of the brain with respect to protective T cell responses (96), and further highlights the formidable obstacles to mounting and sustaining such responses to malignant gliomas. Defects in glioma-associated antigen-presenting cells have also been described, including the down-regulation of MHC class I and class II, B7 and other costimulatory molecules (95,105). Taken together, these findings raise the possibility that the depressed T cell induction as well as reduced effector function associated with gliomas contributes to their dismal clinical outcome.

5. EVIDENCE OF EFFECTIVE ANTI-GLIOMA IMMUNITY

5.1. Evidence of endogenous immune benefits in glioma patients

Despite the evidence that T cell immunity is reduced and that such reduction may worsen clinical outcome in malignant glioma patients, recent indirect evidence also suggests that endogenous immunity may effectively combat glioma growth. Patients with allergies, autoimmune conditions (i.e., pathologic anti-self cellular immune responses), and especially both, for example, were found to be at low risk for developing gliomas, including GBM (106,107). In addition, the case for gliomas eliciting endogenous immune responses against specific antigens was recently substantiated by a report that up to a third of GBM patients harbor IgG (i.e., T cell-dependent) antibodies to the transcription factor SOX6, which is highly overexpressed on glioma tissue, whereas healthy individuals and other cancer patients do not (108). Taken together with data on T cell defects and glioma outcome, these recent studies underscore the possibility that endogenous T cell immunity remains intact, and may have a positive bearing on glioma clinical manifestation and/or outcome.

5.2. 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.

Yu *et al.* conducted the first phase I clinical vaccine trial, which utilized MHC I-eluted peptides from cultured autologous tumor cells pulsed to immature dendritic cells (13). Vaccine was administered in 3 semi-weekly courses to 9 newly-diagnosed high-grade glioma (2 anaplastic astrocytoma, 7 GBM) patients, all of whom had undergone image-complete resection and radiotherapy. Due to the lack of radiographically detectable tumor tissue resulting from image-complete resections in this study, radiographic responses could not be informatively monitored. Four of 7 patients tested exhibited positive CTL response induction. In addition, post-vaccine infiltration of tumor by memory and $CD8^+$ T cells was observed in 2 of 4 re-resected patients, and these 2 appeared to survive longer than their counterparts without $CD8^+$ infiltration during the reported time span. Moreover, although the population size and diversity within this trial precluded the acquisition of statistically significant survival data, there appeared to be a modest improvement in survival compared with historical controls. No serious adverse effects were observed.

Kikuchi *et al.* conducted a phase I trial in 8 recurrent malignant glioma (1 anaplastic oligodendroglioma, 2 anaplastic astrocytoma, 5 GBM) patients, which utilized DC fused to glioma cells (44). This strategy potentially minimizes activation of T cell responses to normal brain antigens that could increase the risk of autoimmunity. Vaccine was administered in 3-7 courses every 3 weeks. Increased anti-glioma responsiveness that in two patients appeared specific to autologous tumor cells was observed after vaccination in 6 patients analyzed. Two "partial" radiographic responses, in which either one portion of tumor regressed while another progressed, or tumor-associated brain edema but not tumor itself was diminished, were seen. No serious adverse effects were observed.

Yamanaka *et al.* conducted a phase I/II trial in 10 recurrent high-grade glioma (1 non-descript, 1 mixed, 1 analplastic oligodendroglioma, 7 GBM) patients (45). Five patients received 2-6 intradermal vaccinations with tumor lysate-pulsed DC, and 5 patients received 1-10 intradermal vaccinations as well as 1-7 intratumoral administrations via Ommaya reservoir of lysate-pulsed DC, at 3 week intervals. Three of 8 patients tested exhibited increased immunological responsiveness to tumor lysate following vaccination. Two partial radiographic responses were observed, in which contrast enhanced tumor images, but not necessarily other tumor regions, were diminished. In addition, no serious adverse effects were observed.

A phase II trial reported by Yu *et al.* targeted 12 recurrent (3 anaplastic astrocytoma, 9 glioblastoma multiforme) and 2 newly-diagnosed (1 anaplastic astrocytoma, 1 GBM) patients, and administered 4 tumor lysate-pulsed DC vaccinations every two weeks (46). As in the first glioma DC vaccine trial, image-complete resections precluded informative monitoring of radiographic responses in this trial. *In vitro* responses against autologous tumor lysate were induced following vaccination in 6 of 10 patients analyzed, and *in vivo* responses against known tumor antigens were observed in 4 of 9 patients analyzed. In addition, evidence consistent with post-vaccine $CD8^+$ memory cell infiltration into tumors was provided. Finally, vaccinated patients appeared to

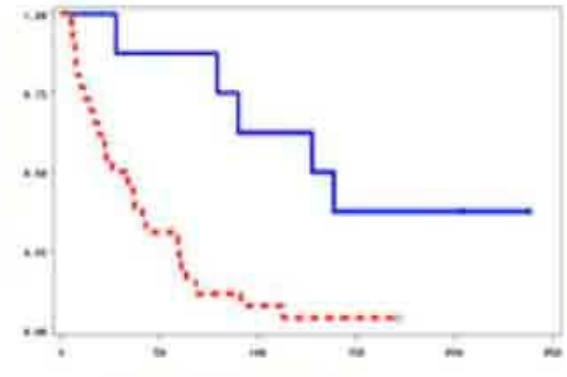

Figure 4. Kaplan-Meier survival curve of DC vaccinated study group (n = 10; solid line), and control group (n = 51; dashed line) of patients with recurrent GBM from time of second craniotomy. The median survival for the study and control groups were 132 and 30 weeks, respectively, for recurrent patients. The Mantel COX log-rank test revealed that the survival curves for the two groups were significantly different, p = 0.003). Of note, 3 patients have survived over 200 weeks. For further details, see (46). Reproduced with permission from the American Association for Cancer Research. Cancer Res (46).

enjoy markedly prolonged survival relative to historical controls in this non-randomized trial (figure 4). This apparent survival enhancement is remarkable, because vaccination of newly-diagnosed GBM patients in the earlier phase I trial from this same institution exhibited only modestly enhanced survival (13). The phase II trial, on the other hand, utilized 25-fold greater numbers of antigen-pulsed DCs than the original, highlighting the possibility of dose-limited vaccine-enhanced T cell responsiveness.

In this last DC therapy trial, it was not possible for the authors to rule out a selection bias favoring inherently longer survival of vaccinated versus control patients independent of their treatment status, particularly as the relevant group of patients was treated at a later point in their disease than patients in the earlier vaccine trial from this same group. This would tend to bias the test population toward inherently longer survival, a common confounding issue in the interpretation of non-randomized clinical trials. In this context, empirical pre-treatment tumor recurrence data have recently been employed to support a likely absence of selection bias among non-randomized vaccinated glioma patient cohorts, and to validate treatment-related affects (109). Similar *post hoc* empirical analysis, which might complement techniques that group glioma patients into outcome categories based purely on statistical trends to minimize selection bias (3), could help resolve whether apparent changes in DC vaccine clinical outcomes are related to actual treatment.

Although differing widely in design, clinical glioma vaccine trials have demonstrated that T cell responses can indeed be induced in high-grade glioma patients, despite concerns over T cell suppression. They also provided fallible evidence that is nonetheless consistent with improved clinical outcomes after vaccination in the highest-grade glioma (GBM) patients.

6. IMPROVING GLIOMA VACCINES

Identifying the cells capable of slowing or halting tumor progression in cancer patients, identifying the critical effector functions of the immune system in counteracting human tumor progression, or both, is 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 (47). 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 (17). The conclusion that arises is that tumor Ag-pulsed DC therapy is sufficient to counteract tumor growth in animal models, but that additional parameters critically limit the ability of human T cells to elicit net tumor destruction.

Autoimmune sequelae have also been observed in cancer patients treated with some forms of immunotherapy (110), raising particular concerns for vaccines for tumors in vital tissues such as the brain. Thus, major challenges facing DC vaccine therapy for cancer patients in general and glioma patients in particular, include diminishing the risk of pathologic autoimmunity, identifying the cellular basis of beneficial anti-tumor immunity, and increasing the proportion of patients experiencing such benefits. Recently developed molecular assays for identifying and quantifying nascent T cell subpopulations and T cell recognition of tumor antigens have proven invaluable in advancing knowledge along these lines.

6.1. Safety & autoimmunity

Although no autoimmune sequelae were evident in DC glioma vaccinated glioma patients, their design allows T cell responses against unidentified antigens that could be expressed on normal brain cells. Coupled with the brain's vital nature, this emphasizes concern over potential pathologic autoimmune reactions against normal brain components following DC therapy in glioma patients. Thus, the need to move toward greater specificity when designing future generations of glioma vaccines is recognized. Progress in this regard has now been realized through analysis of known tumor antigen epitopes on glioma tissue.

The characterization of tumor antigens on gliomas was initially intended as a means to target toxic moieties to tumors using specific antibody-toxin conjugates. A mutant form of EGFR (vIII) expressed on up to 50% of human GBM has been the most vigorously pursued glioma-associated antigen in this regard (111,112).

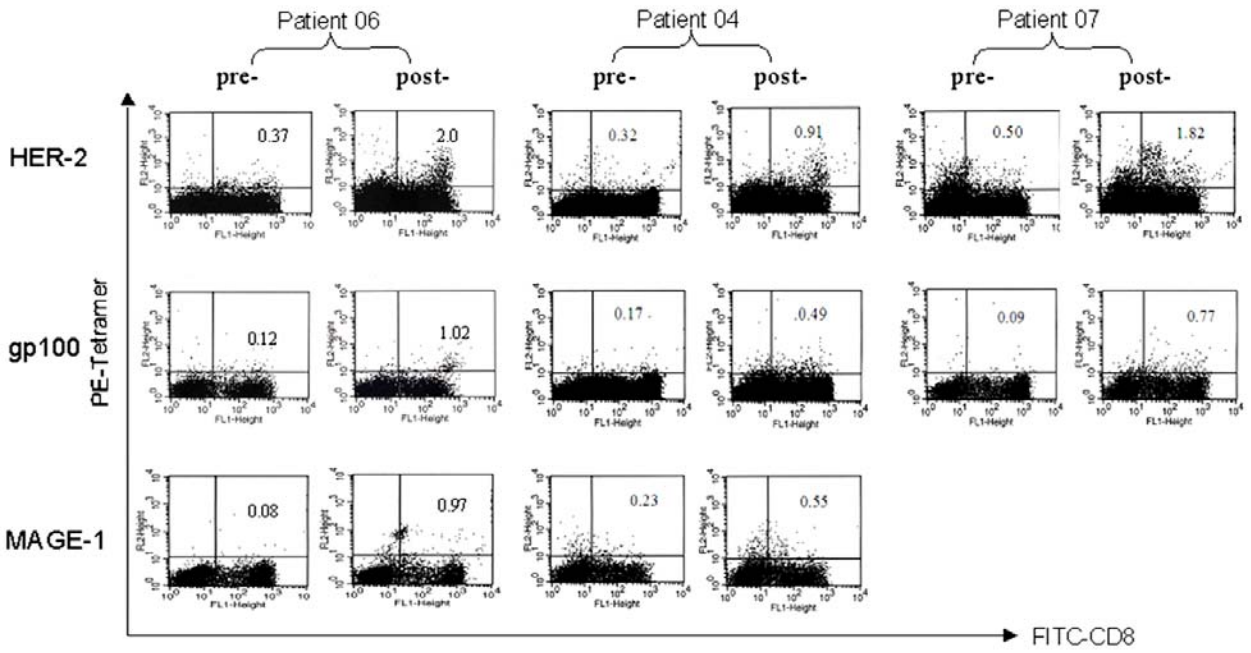

Figure 5. Identification of tumor associated antigen specific T cells in PBMC from four pre- and post-vaccinations. PBMC were stained with HLA-A2 restricted tetramers for HER-2, gp100, and MAGE-1 (y-axis), then cells were stained for the CD8 (x-axis). The number shown in the plots indicate the percentage of TAA specific T cells in whole PBMC population. For further details, see (46). Reproduced with permission from the American Association for Cancer Research. Cancer Res (46).

Attempts to identify and incorporate peptide epitopes from EGFRvIII into therapeutic vaccines for gliomas are also in development (113). An alternative approach, identifying antigens expressed by gliomas that have been demonstrated to mediate immune responses or regression in distinct human tumors (i.e., melanoma), may increase the likelihood of achieving beneficial immune responses upon DC vaccination, and has recently been undertaken.

Examining the expression of melanoma antigen genes on primary cell lines from GBM patients was originally performed to provide evidence of tumor status, and, hence, suitability of such lines as sources of tumor antigen for DC vaccines (13). The first antigens examined, gp100, MAGE-1, and TRP-2, were previously identified in non-glioma tumors, representing two subclasses of tumor antigens: differentiation antigens and cancer/testis antigens (24,25). More recent studies show that GBM patients vaccinated with autologous tumor lysate-pulsed DCs can mount responses directed against epitopes of TRP-2, a melanoma antigen also expressed by gliomas (114), as well as against distinct classes of tumor antigens such as Her-2 (46,102) and AIM-2 (115), in addition to gp100 and MAGE-1 (46)(figure 5). Together with the previously mentioned studies on SOX6 (108), this further demonstrates intact cellular as well as T-dependent humoral immunity in glioma patients, and identifies tumor Ag epitopes that could be useful in increasing glioma vaccine specificity, monitoring immunological endpoints after vaccination, or both. The development of DC vaccines that target these specific epitopes in glioma patients, while potentially increasing the risk of immune resistance due to single epitope loss following vaccination (62,64), should substantially reduce the risk of vaccine-induced autoimmunity against normal brain components. Empirical assessment of the relative impact of these competing risks on glioma vaccine efficacy will determine the practicality of incorporating these epitopes into therapeutic vaccines.

6.2. Fundamental limitations to beneficial cellular immunity in glioma patients.

In glioma DC vaccine trials, as in distinct cancer vaccine trials, vaccination is insufficient to elicit net tumor destruction in a majority of treated patients, and radiological decreases in tumor is either not evident, somewhat subjective, or partial in nature. As a rational approach to improving therapeutic DC vaccines against glioma, a broad consideration of parameters limiting tumor destruction by T cells, built upon recent studies of endogenous and vaccine-induced immunity in glioma patients, is useful. Since animal tumor models may be inaccurate in this regard (17), the nature of such parameters limiting beneficial anti-tumor immunity might best be appreciated from more successful immunotherapeutic approaches in cancer patients. For gliomas, successful therapies of any sort are very rare, and the administration of cytokines or receptor-specific antibodies has generally failed to elicit net glioma destruction (116). A recent clinical trial, on the other hand, has revealed surprising evidence of glioma destruction following adoptive T cell therapy (117).

It has been argued that adoptive transfer of Ag-reactive T cells is consistently more clinically beneficial

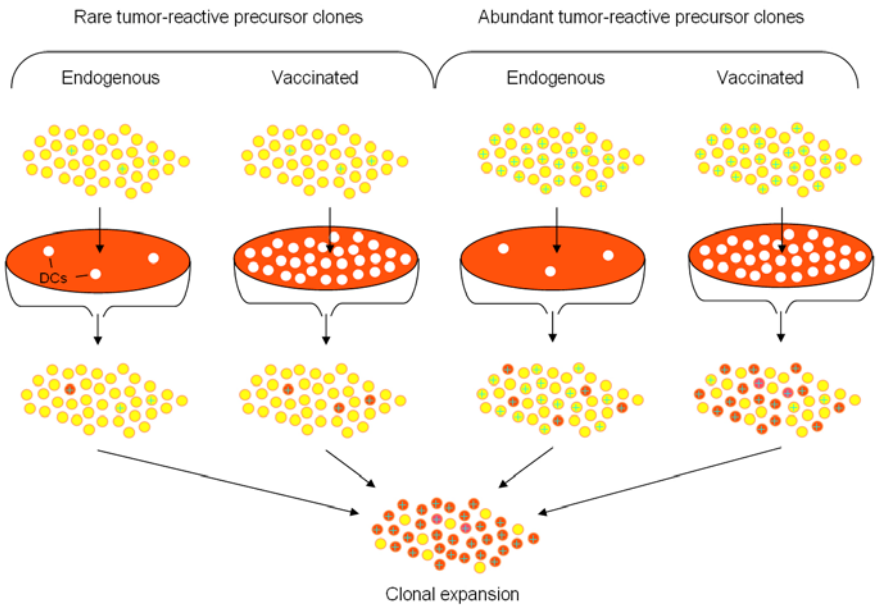

Figure 6. Limiting processes in anti-tumor T cell immunity. Under all conditions, the primary limiting factor in anti-tumor immune induction is interaction between tumor-reactive T cell precursors (yellow circles with blue crosses; presumed to be naïve T cells) and cognate antigen-bearing APCs capable of activating naïve T cells (i.e., DCs; white circles) within local lymph nodes (red ovals) or elsewhere. When DCs are rare, as in the absence of DC vaccination, such interactions are limited by DC presence, and relatively few reactive clones will be activated (red circles with blue crosses). When tumor-reactive T cell precursors are rare, as may be the case in human cancer patients, T cell:DC interactions will be additionally limited by levels of tumor-reactive T cells, also leading to few activated clones. Thus, the activation of maximal numbers of tumor-reactive clones is specifically promoted when both DCs and tumor-reactive T cells precursors are abundant. This model assumes clinically beneficial anti-tumor immunity is dependent upon the activation of maximal numbers of reactive T cell clones, and as such predicts differential immunological and clinical vaccine outcomes between human cancer patients and animal tumor models. In particular, T cell responsiveness, while evident under many conditions, is predicted to correlate with clinical benefits of immunity under this model only when both DCs and tumor-reactive T cell precursors are abundant.

than DC vaccination in cancer patients (118). This argument gained further credibility when Dudley *et al.* found that a majority of melanoma patients enjoyed objective clinical responses following adoptive transfer of *ex vivo*-cultured, tumor-reactive T cells derived from the patients' tumors (110). A series of clinical trials performed previously by this same group, including several DC therapy trials for similar melanoma patients, failed to reach this degree of success (25). In this study, the unique success of adoptive T cell therapy was dependent on a pre-existing population of highly tumor-reactive T cells in cultures, on the number of such reactive cells transferred, and on chemo-ablation of hosts to facilitate homeostatic expansion of the transferred cells (110). These findings parallel the recent glioma adoptive T cell trial, which documents impressive and long-term objective tumor regression by serial imaging studies in up to a third of patients treated with 107-108 adoptively transferred, tumor-reactive T cells (117).

Unfortunately, huge numbers of highly tumor-reactive cultured T cells are required for adoptive therapy, and this fact currently precludes its wider application in glioma patients. In addition, only DC vaccination, a therapy that can be more universally applied to patients with surgically accessible tumors, has hinted of improved survival in high-grade glioma/GBM patients (13,46). Consequently, the true utility of adoptive T cell therapy may stem from its defining critical limitations to beneficial anti-glioma immunity. In this respect, the dependence of immune tumor destruction on the presence of highly glioma-reactive T cells in the host is inferred by the apparent success of this therapeutic approach. In addition, peripheral expansion and/or peak numbers of adoptively transferred T cells *in vivo* appears to limit their clinical efficacy. Critical limitations to beneficial anti-tumor immunity in human cancer patients therefore appear to exist at the level of tumor-reactive T cells. Since tumor reactivity of T cells *per se* does not appear to be unique in humans, this implies that a characteristic associated with greater numbers of tumor-reactive T cells, either before or after clonal expansion, critically limits beneficial anti-glioma immunity.

The notion that beneficial anti-tumor immunity may be limited by numbers of tumor-reactive effector T cell precursors is expected if, for example, clinical benefits of immunity are traceable to a few effector T cell clones exhibiting the strongest interactions against tumors. This appears to be supported by the observation that cancer patients exhibiting strong tumor Ag-tetramer binding include those experiencing clinical benefits (16,119). Powerful DC vaccines, on the other hand, might induce expansion of even a tiny number of tumor-reactive T cell precursor clones, which would not necessarily include those interacting most strongly against tumors (figure 6). Thus, hosts in which tumor-reactive T cell clones were rare

might exhibit anti-tumor immune responses after vaccination, but also a general lack of correspondence of clinical vaccine benefits with such response, as is typical of human cancer patients. By contrast, only hosts with abundant tumor-reactive effector precursor T cell clones would exhibit a consistent correlation between clinical benefits and vaccine responsiveness, as is typical of animal tumor models. The correspondence of this model (summarized in figure 6) to differences in experimental and clinical cancer vaccine dynamics suggests that experimental animal tumor models may generally differ from human cancer patients in harboring substantially greater numbers of tumor-reactive T cell precursor clones. Given that T cell receptor diversity, T cell generation and selection during development, and functional T cell subsets are all broadly similar between humans and animals typically used for tumor models, it is not entirely obvious why such a putative discrepancy in tumor-reactivity may exist. Nevertheless, the possibility that tumor-reactive effector precursor T cells as a group are specifically deficient in human cancer patients is raised by this working model.

Based on the above considerations, beneficial anti-tumor T cells as a group are expected to possess intrinsically high tumor-reactivity, to be limited in number prior to vaccination in cancer patients, and ample in rodents used for tumor studies. Moreover, these cells are expected to confer clinical benefits according to both their pre-vaccine numbers and the degree to which they expand *in vivo* post-vaccination. Finally, such cells as a group are expected to possess enhanced avidity to tumor antigen-bearing tumor targets. These points are expected to provide a template guiding the analysis of discrete T cell populations that may dominantly mediate beneficial anti-tumor responses.

6.2.1. The thymus and anti-tumor immunity

Hints as to the nature of a discrete group of T cells that might critically limit beneficial anti-glioma immunity follow from the appreciation that numbers of highly tumor-reactive T cells, as well as diversity within responding clones of cells, depend upon randomly-generated T cell receptor (TCR) specificities. Since each T cell is normally capable of recognizing and responding to one or a limited set of related antigenic epitopes, the existence of an antigen-specific T cell is therefore dependent upon a fairly high level of T cell (TCR repertoire) diversity. Hence, the level of T cells specific for all tumor epitopes should also be dependent on a certain level of TCR repertoire diversity, with greater repertoire diversity conferring greater capacity to respond to multiple unrelated tumor epitopes.

The production and emigration of nascent, thymus-derived T cells (recent thymic emigrants, or RTEs) is critical to the maintenance of TCR repertoire diversity in most vertebrates, and T cell diversity declines dramatically as thymus production of RTEs declines later in life (120-122). Thus, T cells from older patients, being relatively deficient in RTEs, might have decreased capacity to respond to tumor epitopes. This expected (and actual) decline in RTE production with age closely parallels the strongly age-dependent progression of high-grade gliomas, including GBM (102,123). In addition, RTEs themselves are expected to be less subject to immune suppression due to their nascent status. RTE survival signaling and homeostatic expansion is also uniquely exempt from competitive interference by other T cells (124), potentially minimizing constraints on the *in vivo* expansion of RTEs similar to those impeding the expansion of adoptively transferred T cells. This exemption could also conceivably minimize susceptibility of RTEs to signaling molecule defects as well, since such defects appear to follow from limited local access to T cell survival ligands (61). The possibility that RTEs might represent a unique pre-tolerized T cell subpopulation that might be uniquely reactivity to tumor antigens was also considered. Finally, recent studies suggest that RTE levels, particularly those of $CD8^+$ RTEs, may be 10-fold lower in healthy human subjects relative to experimental rodents (125,126). Such speculative considerations tend to favor the view that RTEs may be uniquely competent to initiate or sustain anti-tumor responses in patients, and thus encourages the hypothesis that RTEs play a significant dose-limiting role in beneficial anti-glioma immunity in patients.

The finding that $CD8^+$ RTEs are specifically enriched within tumor-infiltrating T cells, as peripheral RTEs decrease, in glioma-bearing rats (103,127), adds weight to the notion that $CD8^+$ RTEs as a group are dominantly involved in beneficial anti-glioma immunity. 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).

6.2.1.1. $CD8^+$ RTE activity & dominance

Based on the above considerations, 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 (122,128), the presence and post-vaccine responsiveness of $CD8^+$ RTEs was found to not only accurately predict age-dependent clinical outcome in GBM patients generally, but to largely account for the influence of age, the strongest established prognostic factor, on such outcome (figure 7)(102). In addition, glioma-bearing mice specifically deficient in thymic production of $CD8^+$ RTEs, but not peripheral $CD8^+$ T cell activity, exhibited the decreased age-dependent survival and strong correlation between thymic cell production and clinical outcome observed in human GBM patients (figure 8). 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 (102). 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 (102). Moreover, the vast majority of T cells

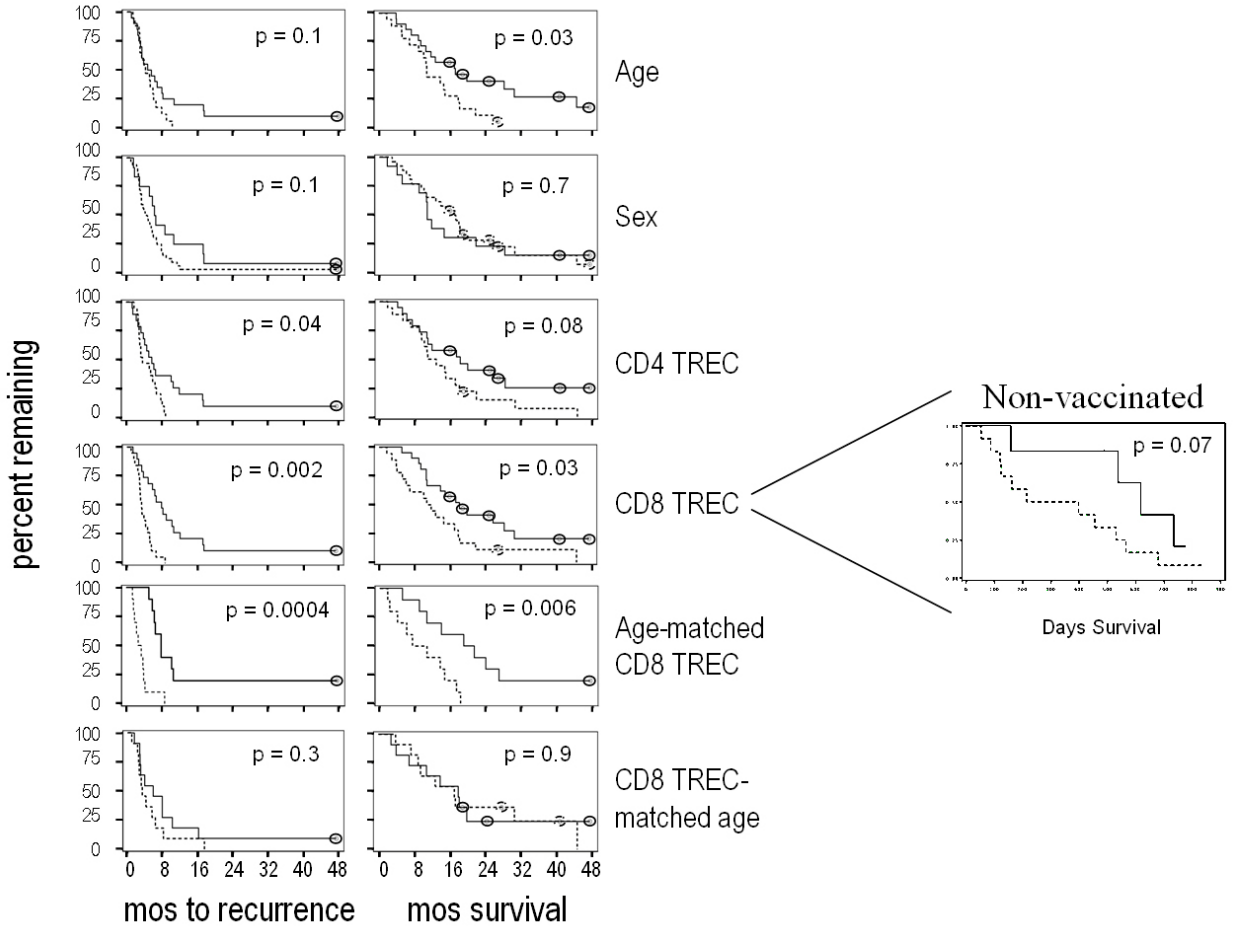

Figure 7. TRECs within purified T cells, a molecular parameter quantifying RTE levels, account for age-dependent GBM outcome. Patients were separated for analyses by the indicated parameters above or below their median values in the entire population, in CD8 TREC-matched cohorts, or in age-matched cohorts 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 $CD8^+$ TRECs (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 $CD8^+$ TRECs ($P < 0.05$) or age ($P < 0.008$), respectively. Expansion to right depicts contribution of non-vaccinated patient subgroup to ability of CD8 TRECs to predict survival. 2-tailed Mann-Whitney log-rank tests for disease-free and overall survival were calculated with SAS software. Modified from (102). Reproduced with permission from the Thomson Corporation. Current Opin Mol Ther (129).

binding any of 4 distinct tumor Ag/HLA multimers exhibited a $CD8^+$ RTE phenotype, and related activated cells were 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 the $CD8^+$ RTE subpopulation may possess intrinsically high tumor reactivity, that levels of these cells are limited prior to vaccination in many GBM patients, and that 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 both directly mediate and critically limit beneficial anti-glioma immune responses, and appear to dominate over other responding T cells in this regard upon DC vaccination. This emerging knowledge affords potentially extraordinary opportunities to both rationally improve existing DC-based therapies, as well as to probe immune influences on established glioma characteristics in patients.

We propose that existing DC-based vaccine therapies for glioma can be rationally improved by increasing the production of normally rare $CD8^+$ RTEs in patients. Alternatively, determining the salient features of $CD8^+$ RTEs that afford them the apparent ability to dominantly influence glioma progression may allow the transfer of greater tumor-destructive capacity onto more readily available T cells. In this context, the salient feature of $CD8^+$ RTEs that affords these cells apparently greater responsiveness to tumor antigens appears to be evident at the level of tumor antigen/HLA recognition, as evidenced by their dominant role in binding tumor antigen/HLA

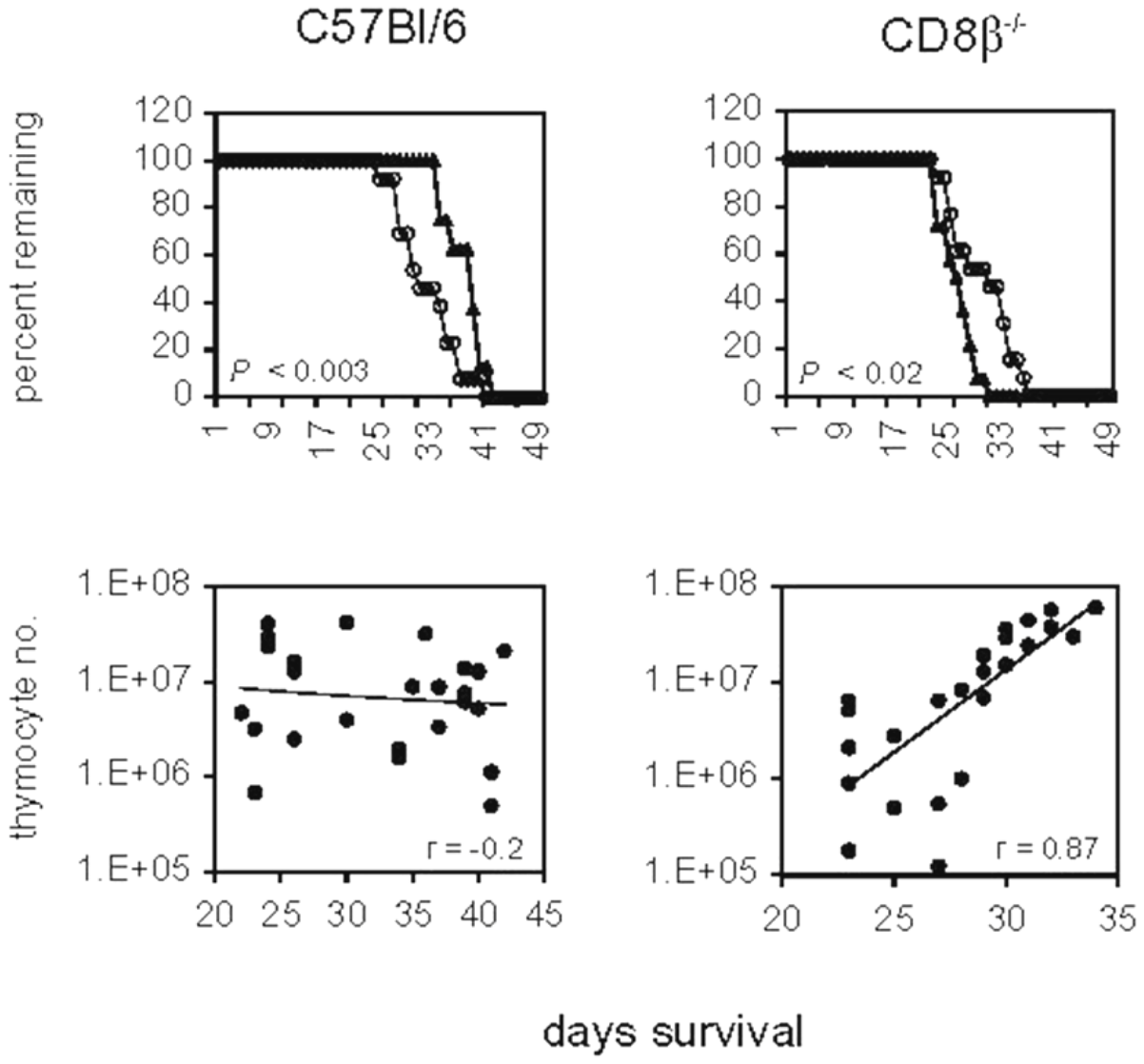

Figure 8. Decreased thymic $CD8^+$ T cell production in $CD8\beta^{-/-}$ mice limits glioma progression in an age-dependent manner. $CD8\beta^{-/-}$ mice are specifically deficient in thymic production, but not peripheral activity, of $CD8^+$ T cells. Top row: Intracranial tumor cell implantation into younger (open circles) and older (solid triangles) wild-type C57Bl/6 or $CD8\beta^{-/-}$ mice reveals uniquely decreased survival in older $CD8\beta^{-/-}$ mice ($P < 0.02$; Mantel-Cox log rank). Bottom row: Thymocyte numbers were determined in all glioma-bearing wild-type C57Bl/6 or $CD8\beta^{-/-}$ mice, and correlated (Pearson's coefficients) with survival time. Strong correlation similar to that observed between $CD8^+$ RTEs and GBM patient clinical outcome ($r = 0.86$; $P < 0.001$ in both cases) was observed exclusively in $CD8\beta^{-/-}$ mice. For further details, see (102). Reproduced with permission from the American Association of Immunologists. J Immunol (102).

multimers (102). This points to possible modifications of antigen/HLA receptors (i.e., TCR and/or CD8) on $CD8^+$ RTEs that may render them more cognitive of and reactive to tumor antigens. These putative modifications are also expected to diminish with the further maturation of $CD8^+$ RTEs in the periphery. Indeed, we have detected temporally restricted modifications specific to both human and murine $CD8^+$ RTEs (CJ Wheeler, unpublished data). Examining these critical modifications on $CD8^+$ RTEs may provide an increased understanding of tumor antigen responsiveness by T cells, how to increase such responsiveness in more abundant T cells, and of critical tumor-immune interactions generally.

6.3. Immunoediting & 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

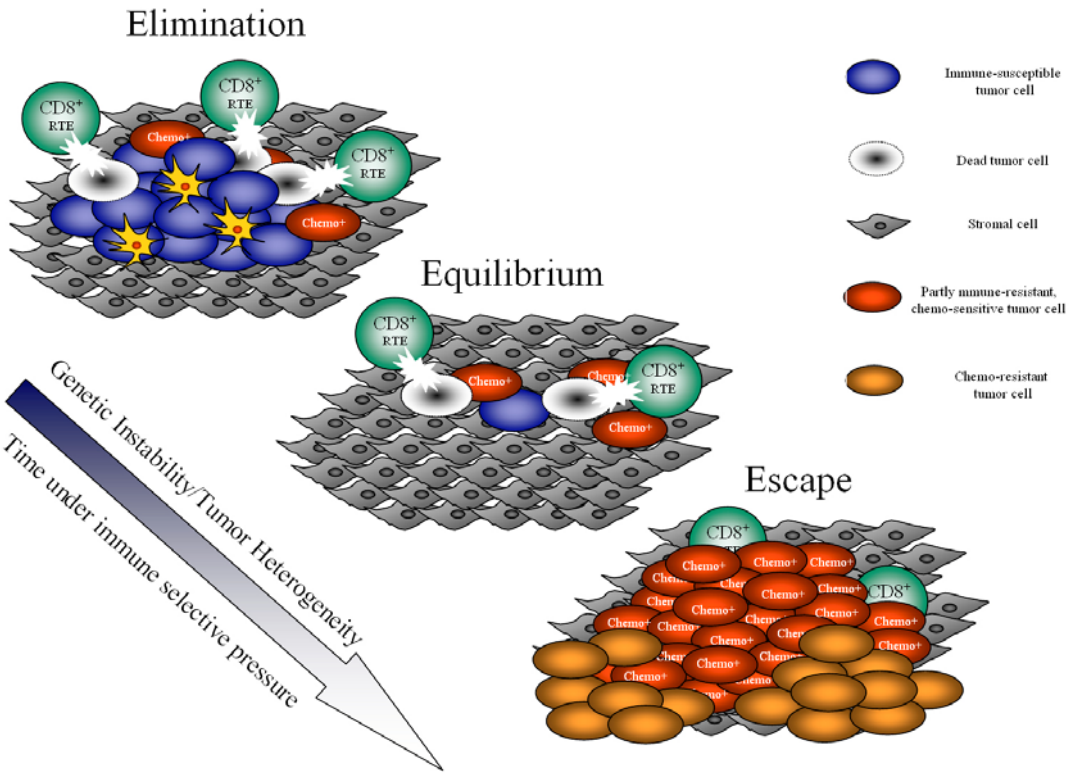

Figure 9. The Immunoediting model in the context of glioma-immune dynamics. Tumor cells susceptible to destruction (blue) by immune cells (activated $CD8^+$ RTE progeny; green) initially grow interspersed with normal (gray) cells in the brain, leading to antigen uptake by nearby antigen-presenting cells (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. Adapted from (132). Reproduced with permission from the Thomson Corporation. Current Opin Mol Ther (129).

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 (62-64). 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 (130,131). 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 the 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 outcome in tumor patients. 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.

Concepts pertaining to the interaction between tumor and immunity 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 (132) updates the earlier concept of immune surveillance, wherein host immunity prevents the development of nascent tumors, and thereafter becomes irrelevant (133). This new model, substantiated by a growing body of experimental evidence (131,132,134), holds that tumors experience 3 distinct phases of interactions with host immunity: elimination, equilibrium, and escape (figure 9). Elimination refers to the complete immune-mediated destruction of nascent tumor cells prior

to their establishment, essentially embodying the original immune surveillance hypothesis (131,133). 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. Although little direct evidence exists to suggest such a possibility, one recent study whose results are outlined below may afford a unique opportunity to gain insight into, and possibly exploit, glioma immune resistance in just such a manner.

6.4. 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 (109).

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 (109). 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 10). Multiple patients also exhibited objective (>50%) regression of tumor burden, an extremely rare phenomenon in GBM (figure 10). 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 (8). 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 11).

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 (135). 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 9). Because chemosensitivity of gliomas, including GBM, has been linked to tumor genetics (136), superimposition of these data onto the immunoediting model suggests that immune selection may drive *in situ* glioma evolution away from a chemo-resistant genotype, and toward a chemosensitive one (figure 9). An equally valid alternative notion, that post-vaccine chemotherapy enhances anti-tumor immune responses by selectively killing suppressor T cells, is inconsistent with recent data that suggests the induction of genetic abnormalities associated with glioma chemosensitivity after DC vaccination (CJ Wheeler, KL Black, unpublished data).

The result of combining therapeutic vaccination with genotoxic therapies may be to increase the proportion of patients experiencing clinical vaccine benefits, in addition to increasing the apparent magnitude of such benefits. Since age is the single most dominant factor influencing the outcome of most human tumors, it will be additionally important to determine whether cellular immune processes similarly influence clinical outcome and chemotherapeutic efficacy in distinct human tumors. If so,

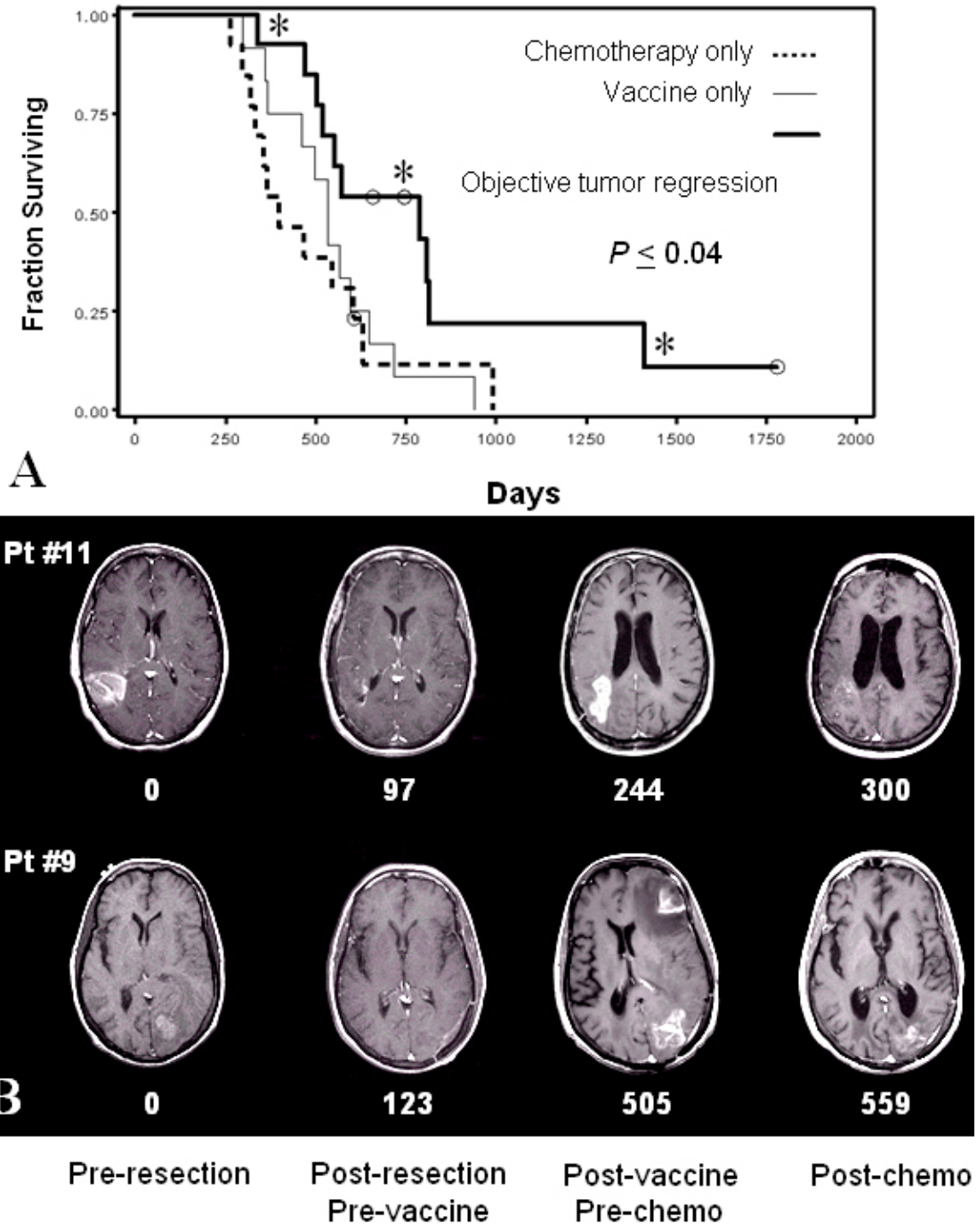

Figure 10. A: Overall survival in patients receiving vaccine, chemotherapy, or vaccine + chemotherapy. Overall survival was defined as the time from first diagnosis of brain tumor (*de novo* GBM in all cases) to death due to tumor progression. Kaplan-Meyer survival plots with censored values in open circles are shown for each group. Survival of the vaccine group was identical to that of chemotherapy group ($p = 0.7$, log-rank). Survival of vaccine + chemotherapy group was significantly greater relative to survival in the other two groups together ($p = 0.048$, log-rank), greater than survival in the chemotherapy group alone ($p = 0.028$, log-rank), and greater than survival in the vaccine group alone ($p = 0.048$, log-rank). B: Tumor regression following post-vaccine chemotherapy. Days after diagnosis are represented by numbers under individual MRI scans, with individual patients' scans in each row. All scans except the pre-resection scan for patient #2 were performed post-contrast enhancement with gadolinium. For further details, see (109). Reproduced with permission from the American Association for Cancer Research. Clin Cancer Res (109).

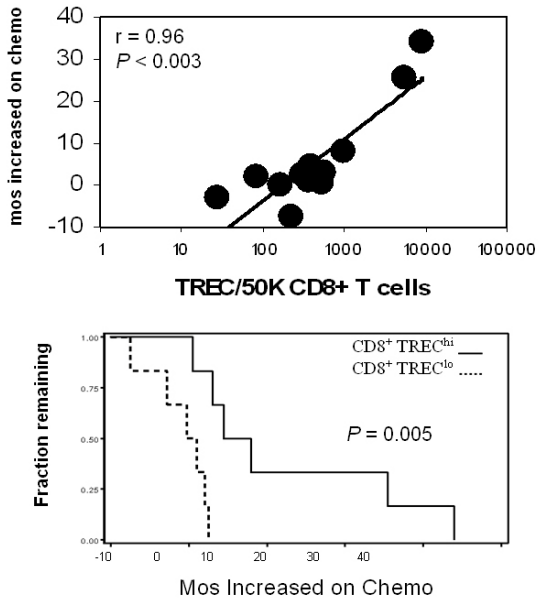

Figure 11. Levels of nascent $CD8^+$ T cells ($CD8^+$ recent thymic emigrants, or $CD8^+$ RTEs) are strongly associated with chemotherapeutic responses following vaccination. TRECs, a molecular measure of RTEs, within purified $CD8^+$ T cells collected at the time of surgery were correlated with the 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. Modified from (109). Reproduced with permission from the Thomson Corporation. Current Opin Mol Ther (129).

the clinical expectations associated with immune-based cancer therapies would be substantially broadened.

7. SUMMARY AND PERSPECTIVE

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 (13,44-46). Impressive clinical responses have been observed, but in general these still occur in small subgroups of patients (16,37,42,109). In addition, and unlike in rodent tumor vaccine models, clinical improvement in vaccinated cancer patients does not generally coincide well with anti-tumor memory T cell responses (47). 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 (17). 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.

We now know that the induction of T cell responses against autologous tumors is possible through the administration of unfractionated antigen-pulsed or tumor-fused DCs in high-grade glioma patients, including GBM, and that this proceeds without serious autoimmune sequelae given the current natural histories of these cancers. This validates evidence that endogenous immunity is intact, potentially protective, and can be enhanced in glioma patients, while opening the door to the development of more specific and optimized glioma vaccines. A number of candidate antigens expressed by gliomas that could be useful in this regard have now been characterized, including EGFRvIII, Her-2, MAGE-1, TRP-2, gp100, AIM-2, and SOX6. Clinical application of epitopes derived from these antigens will follow demonstration of their efficacy in animal vaccine models.

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. Specifically, the finding that $CD8^+$ T cell production underlies age-dependent glioma clinical outcome suggests that particular patient subgroups based on age and/or $CD8^+$ T cell production should benefit preferentially from vaccination strategies that aim to activate such T cells. Ongoing studies aimed at identifying such subgroups will allow more efficient targeting of vaccines to the patients most likely to benefit from them. In addition, the same finding suggests that increasing newly produced $CD8^+$ T cells or otherwise conferring their relevant anti-tumor properties on greater numbers of $CD8^+$ T cells in glioma patients will enhance therapeutic responses and patient survival after vaccination. This concept is undergoing testing in animal models.

No single treatment modality is likely to effectively eliminate gliomas over long time 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. Taken a step further, selectively opening the blood-brain tumor barrier with vaso-modulators after vaccination might further enhance this synergistic therapeutic approach. Similarly, neural stem cells can be designed and administered to optimally complement immune-based therapies, or alternatively designed to overcome limitation inherent to immune-based therapies. Independent approaches, such as focal irradiation or microwave ablation of tumors, are also particularly intriguing when considering immune-synergistic glioma therapies. It is our hope that such concerted therapeutic efforts will ultimately lead to the diminished need for open craniotomies and increases in both quality of life and lifespan for high-grade 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 success 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 chemosensitivity 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.

To be sure, general issues that hamper objective interpretation of clinical vaccine success in cancer patients require much attention as well. Universally optimized standards for the design or monitoring of DC therapy and other vaccines have not been established. These shortcomings are expected of a therapeutic modality still in development, and measures discouraging this situation should be adopted as outcomes from cancer vaccine trials improve. In this respect, the study of glioma patient immunity and immunotherapy will necessarily follow trends set in more extensively investigated tumor systems. On another level, however, the rapid progression and short clinical histories of high-grade gliomas, their relatively confined, non-metastatic nature, and the existence of clear demographic predictors of disease outcome have allowed the study of these tumors – and particularly that of GBM - to contribute uniquely to our knowledge of beneficial immunity in cancer patients. These properties should continue to favor the analysis of tumor-immune interaction dynamics in gliomas, the results of which promise to improve therapies for malignant glioma and other cancer patients.

8. ACKNOWLEDGEMENT

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Abbreviations: GBM: glioblastoma multiforme, RTE: recent thymic emigrant, CNS: central nervous system, BBB: blood-brain-barrier, CTL: cytotoxic T lymphocyte, MHC: major histocompatibility complex, Ag: antigen, TCR: T cell receptor, Th: T helper cell, DC: dendritic cell,

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