Expert Review of Neurotherapeutics

ISSN: 1473-7175 (Print) 1744-8360 (Online) Journal homepage: informahealthcare.com/journals/iern20

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Maciej S Lesniak

To cite this article: Maciej S Lesniak (2006) Immunotherapy for brain tumors: *quo vadis*?, Expert Review of Neurotherapeutics, 6:4, 447-451, DOI: 10.1586/14737175.6.4.447

To link to this article: https://doi.org/10.1586/14737175.6.4.447

Maciej S Lesniak
The University of Chicago, Neurosurgery, MC 3026–5841 S. Maryland Avenue, Chicago, IL 60637, USA
Tel.: +1 773 834 4757
Fax: +1 773 834 2608
mlesniak@uchicago.edu

Immunotherapy for brain tumors: *quo vadis*?

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Expert Rev. Neurotherapeutics 6(4), 447–451 (2006)

At a recent international brain tumor advisory meeting held in Scottsdale, AZ, USA the topic of immunotherapy for brain tumors was hotly debated. After an intriguing presentation highlighting the most recent attempts at creating a successful vaccine, one of my esteemed colleagues asked a poignant question: "it would appear to me" he said, "that we have been listening to similar presentations for the last 10 years, yet there really has not been any significant clinical efficacy shown with this approach – where are we heading?" Clearly, this statement struck at the heart of those of us involved in developing novel immunological approaches for malignant brain tumors. Whether our work was called into question, or some of our egos were bruised, the fact remains that our success in this arena has been limited. This is not to say that we have not evolved and advanced our understanding of the role that the immune system plays in the CNS; in fact, the last 10 years has seen an increasing number of clinical trials involving immunomodulatory cytokines, dendritic cells (DCs), and gene therapy vectors, all of which have advanced our knowledge and understanding of brain tumor immunology. However, clinical efficacy has lagged behind these endeavors and therefore, the author thought it would be appropriate to bring us all up to date, and explore the potential benefits and limitations of brain tumor immunotherapy.

The field of tumor immunology, especially as it relates to the CNS, has undergone a significant evolution. The brain has historically been viewed as immune privileged, meaning that it is somehow protected from the immune system. The presence of a blood–brain barrier, lack of organized lymphoid tissue or significant lymphatic drainage, poor major histocompatibility complex (MHC) expression in normal brain parenchyma, and presence of immunoregulatory factors all contribute to the perception of immune privilege. While the above properties contribute to the distinctive immunoreactivity of the CNS, they do not render it completely privileged. They do, however, necessitate an understanding of intricacies of the immune system as it relates to CNS antitumor response.

Cell-mediated responses to neoplasms in the CNS require efficient antigen presentation. The precise cell type and mechanism responsible for antigen presentation in the brain has been debated; however, the ability to ingest or phagocytose foreign antigens, expression of MHC complexes, and the ability to induce T-lymphocyte responses, are all critical to an effective antitumor response. Possible antigen-presenting cells (APCs) in the CNS include astrocytes, endothelial cells, capillary pericytes, DCs and microglia. However, since the 1990s, microglia have emerged as the most likely candidates for antigen presentation in the brain. In addition to their hematopoietic origin, microglial cells possess many characteristics that APCs require, including the expression of MHC class I and II molecules and the expression of costimulatory molecules.

10.1586/14737175.6.4.447

ISSN 1473-7175

In addition to the issues related to antigen presentation, a second potential obstacle to CNS tumor reactivity is the relative lack of MHC expression and antigenicity in normal and malignant brain tissue. However, there is now experimental evidence that MHC antigens are upregulated at sites of brain injury, degenerative disease, tumor, or after exposure to cytokines, such as interferon (IFN)-γ. In addition to antigen-presentation complexes, most human glioma cells have been shown to express Fas/Apo-1 and Fas ligand, which allow these cells to undergo Fas/Fas-ligand interactions with activated T lymphocytes, resulting in apoptosis. While no universal glioma-specific antigen presentation on these MHC molecules has been found, several tumor-associated antigens shared by histogenetically related tumors have been identified (e.g., tenascin, glycoprotein [gp]240, gp1000, epidermal growth factor receptor, tyrosinase, tyrosinase-related proteins 1 and 2, interleukin [IL]-13α2R, melanoma-associated gene [*MAGE*]-1 and *MAGE*-3).

Besides evading host immune responses, malignant brain tumors actively suppress the local immune environment. T-lymphocyte function in the setting of primary intracranial tumors has been shown to be defective with abnormal T-cell receptor-mediated signaling and a depressed proliferative response to mitogen. Moreover, tumor-infiltrating lymphocytes exhibit a propensity for apoptosis. The partial reversibility of T-cell suppression following brain tumor removal along with the demonstration of downregulated function of T cells harvested from brain tumor patients or normal patients exposed to glioma supernatants, provides strong evidence of glioma-produced immunosuppressive factors. Transforming growth factor (TGF)-β_2 has been one of the most extensively studied of all immunosuppressive factors identified thus far. It has been shown to be significantly overexpressed in patients with glioblastoma multiforme, while virtually absent in normal brain tissue. Additionally, TGF-β_2 has been shown to effectively limit T-cell and B-cell proliferation, IL-2 receptor induction, cytokine production, natural killer (NK) cell activity, cytotoxic T-lymphocyte development, lymphokine-activated killer (LAK) cell generation and the cytotoxic response of tumor-infiltrating lymphocytes. Other factors produced by glioma cells that may play a role in immune regulation and tumor cell escape include prostaglandin (PG)E_2 and IL-10.

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In spite of the unique anatomical and physiological barriers posed by the CNS, there is clear evidence that priming of T cells to intracranial tumor antigens does occur although it can be significantly augmented with local immunotherapy. These data led to the investigation of various types of peripheral vaccinations as treatment strategies for brain tumors. First and foremost among them was the development of cytokine-expressing vaccines. Cytokines play critical roles in CNS tumor immunology by enhancing T-cell activation and MHC antigen expression. Cytokine gene transfer in cancer models, resulting in tumor rejection, has been demonstrated with IL-2, -4, -12, -18 and -23, IFN-α and granulocyte–macrophage colony-stimulating factor (GM-CSF). The use of peripheral, cytokine-secreting vaccines for intracranial cancers has, therefore, been explored in the hope of overriding the tumor-induced immunosuppressive microenvironment and enhancing the activation and infiltration of host-derived APCs and leukocytes.

Several studies have demonstrated the efficacy of peripheral vaccinations with GM-CSF-transfected tumor cells against brain tumors in animal models [1–9]. Indeed, some studies have suggested intensified antitumoral responses when GM-CSF vaccines were coupled with those expressing IL-4 or the costimulatory molecule B7-2. In this setting, antitumor cytotoxicity only exceeded that of peripheral blood mononuclear cells (PBMCs) stimulated with wild-type tumors alone, when PBMCs were stimulated with both wild-type tumor and B7-2/GM-CSF-transduced cells. Finally, peripheral tumor cell inoculation coupled with continuous, peripheral GM-CSF alone or with IL-2 or IL-12 resulted in improved survival rates in the treatment of intracranial glial tumors in rat models. It is postulated that the antitumor activity of GM-CSF is related to its potency in generating DCs. Whether GM-CSF alone or in combination with other factors will result in CNS antitumor efficacy in humans remains to be seen.

The utility of IL-2 in the treatment of brain tumors has been investigated using multiple methods [10–17]. IL-2-secreting autologous fibroblasts injected into glioma cells have been demonstrated to induce cytotoxic $CD8^+$ T cells in the blood of a patient with malignant glioma. Intracerebral injection of IL-2-secreting fibroblasts has also been found to be effective as a protective treatment in preventing the development of murine brain tumors when the tumor cells are introduced into the same site where the fibroblasts were injected earlier. Of note, this treatment has proven effective for metastatic brain tumors, including established intracerebral breast carcinoma. Combining peripheral vaccination and intratumoral injection of IL-2-expressing cells or IL-2-activated NK cells has also shown promise in the elimination of brain tumors in animal models. Also, injection of IL-2-generated LAK cells into tumor resection cavities of humans with histologically confirmed recurrent glioblastoma multiforme (GBM) was found to be safe and resulted in higher median survival rates than those reported in other published series of patients that underwent re-operation for GBM. Given the promising results of intracranial IL-2 treatment, novel means of delivery (including the use of biodegradable microspheres) have also been explored. The use of microspheres for delivery of IL-2 and the chemotherapeutic agent carmustine (BCNU) has demonstrated improved median survival when compared with controls in animal models.

The effectiveness of IL-4 for the treatment of intracranial tumors has also been explored in multiple studies [18–20]. The effectiveness of various cytokines (IL-4, IL-12, GM-CSF and IFN-α) has been compared using intracerebral or intradermal injections of transfected tumor cells, as prophylaxis or therapy against intracerebral glial tumors in an animal model. However, only intradermal IL-4-expressing cell vaccinations were shown to be effective at providing immunity against later challenges with intracranial tumors. Additionally, only IL-4-vaccinated animals showed long-term survival benefits when used as therapy for existing tumors. These results corroborated data demonstrating the efficacy of subcutaneous tumor cell vaccines expressing IL-4 (with and without herpes simplex virus-thymidine kinase [HSVtk]/ganciclovir) as therapy or prophylaxis against intracranial glial tumors in animals. Recently, a single patient with recurrent right temporal GBM was vaccinated with autologous glioma cells admixed with transgene-derived IL-4-expressing fibroblasts. A transient response to the vaccine was suggested in this trial and the patient survived for 10 months after treatment. Clearly, further trials with peripheral IL-4 vaccines will be needed to better assess their effectiveness.

The subcutaneous immunization of rats with IL-12-secreting glial tumors has been demonstrated to suppress the growth of identical cell type brain tumors [21–26]. The use of intracerebral cytokine delivery has also been explored by other means. Significantly prolonged survival times have been demonstrated in animals challenged intracranially with glioma cells after earlier intracranial paracrine delivery of IL-12 with transduced glial cells. Neural stem cells (NSC) that express IL-12 have also demonstrated effectiveness in prolonging survival time in mice and rats with existing tumors. Finally, systemic IL-12 has also demonstrated effectiveness in eliciting immunological responses against transplanted and endogenous murine CNS tumors.

> '...direct intratumoral DC therapy appeared to be more effective than peripheral subcutaneous vaccination at generating tumor regression...'

Most recently, DC-based vaccines have received a significant amount of attention. The rationale for using DCs lies in their potency as APCs. With the advancement of techniques allowing for their isolation and propagation *in vitro*, these therapies have become more feasible in the treatment of gliomas. Each of these therapy regimens, however, requires patient-derived tumor antigens, due to the lack of a universal glioma-specific antigen. There are several reports of peripheral vaccinations with DCs loaded with glioma antigens (e.g., tumor cell extracts, lysates and RNA) or DCs fused with glioma cells (with or without recombinant IL-12) leading to the generation or augmentation of antitumor responses in the brains of animal models [27–30]. The promising results of glioma DC vaccines in animal models have led to several clinical trials in adults and children [31–36]. Initially, Phase I trials established the feasibility and safety of peptide-pulsed DCs vaccinations. Most recently, vaccination with tumor lysate-pulsed DCs was shown to elicit the generation of antigen-specific, cytotoxic T cells in patients with malignant gliomas. In this study, the median survival for patients with recurrent glioblastoma who were treated with the vaccine was extended to 133 weeks. Together, these results suggest that DC vaccinations are safe and can generate intracranial antitumor effects and T-cell infiltration.

Although most DC vaccines are administered peripherally, direct intracranial injection with DCs has also been explored in glioma [37–39]. Importantly, preliminary studies indicate that such direct intratumoral DC therapy appeared to be more effective than peripheral subcutaneous vaccination at generating tumor regression. This result was mediated by the migration of DCs to extracranial lymph nodes and resulted in systemic antitumor immunity against intracranial glioma cells. Furthermore, the activity of IL-12 or IL-18 appears to enhance the effectiveness of intratumoral injection of DCs. These results suggest that augmenting the local immune response with cytokines can further enhance the infiltration of T cells into the tumor parenchyma, thereby promoting the interaction of T cells with tumor-pulsed APCs.

Nevertheless, the early success of DCs as vehicles for future vaccine development has to be viewed in the context of potential obstacles. Namely, several problems remain to be solved in order to fully optimize this form of therapy. These include:

- Sources of DCs or precursor cells
- Methods of maturation
- Antigen-loading strategies
- Route of administration
- Role of administering additional cytokines and end points for clinical trials

To date, the major sources of DCs include direct isolation from peripheral blood or *in vitro* generation. A direct comparison of these DCs has not yet been made. Moreover, given the different immunological properties of immature versus mature DCs (antigen uptake and presentation, MHC expression, migratory capacity, expression of adhesion molecules or costimulatory activity), it will be important to determine which subset provides for optimal vaccine. Similarly, the elucidation of the best antigen source, (i.e., peptides, proteins, DNA, mRNA, cell lysates, apoptotic bodies and fusions) awaits further investigation. While most current trials employ subcutaneous or intratumoral immunization, the availability of other strategies, such as intravenous, intradermal or intranodal, intralymphatic, has not been compared, with or without the use of adjuvant cytokines. Finally, there are no established criteria for measuring the success of vaccine therapy, although an increase in overall survival is commonly used as a study endpoint. Perhaps a better determinant of success would be a delay in time to progression or recurrence, given that immunotherapeutic applications are most likely to be successful in clinical situations where the volume of residual disease is low.

Finally, the current literature provides ample evidence for successful use of viral vectors for gene therapy of cancer. For example, HSV has been used to deliver IL-4, IL-10 and IL-12

against experimental glioma [40–43]. The combination of HSV/IL-4 was shown to augment the oncolytic effect of HSV alone and improve the survival of mice with experimental brain tumors. Likewise, local expression of IL-12 via HSV was shown to increase T helper 1 response and significantly increase the survival of mice with intracranial tumors. The effects observed with HSV have been documented with adenoviral vectors for the delivery of IL-4, IL-12 and tumor necrosis factor (TNF)-α and vaccinia-mediated delivery of IL-2 and IL-12. In all instances, mice intracerebrally implanted with viral vectors producing IL-2, IL-4, IL-12 or TNF-α survived significantly longer than those implanted with noncytokine-secreting vectors. These results further confirm the use of local cytokine delivery against brain tumors and, when used in combination with oncolytic therapy, suggest a powerful means for targeted brain tumor therapy.

The development of novel biological therapies for primary brain cancer has been and continues to be intensely investigated. There is abundant evidence that tumor-specific immunity can be induced in both experimental models and patients. However, there is also increasing evidence that as the tumor grows, the ability of the immune system to overcome the cancer fades. On an intuitive level, this makes perfect sense. Historically, vaccines were developed to prevent disease. We are now proposing to cure cancer. As many immunologists probably recognize, the degree of T-cell priming as well as the number of antigen-specific T cells, is probably far below the level that is required to eliminate solid tumors. Targeted vaccines to low-burden disease as well as newer vaccine technologies administered alone or in combination with neoadjuvant disease will certainly optimize clinical results. The question is no longer where are we heading, rather, how will we get there? The important studies in the next few years will analyze immunological approaches to brain cancer not only via clinical response but also via the mechanism of action. Optimizing both arms will likely advance immunotherapy into clinical practice and make a significant impact on the lives of patients with malignant brain tumors.

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Affiliation

- *Maciej S Lesniak*
The University of Chicago, Neurosurgery, MC 3026–5841 S. Maryland Avenue, Chicago, IL 60637, USA
Tel.: +1 773 834 4757
Fax: +1 773 834 2608
mlesniak@uchicago.edu