Tumor immunology, immunomics and targeted immunotherapy for central nervous system malignancies

Haumith R. Khan-Farooqi*, Robert M. Prins* and Linda M. Liau*†‡

*Department of Neurosurgery, †the Jonsson Comprehensive Cancer Center and ‡the Brain Research Institute, David Geffen School of Medicine at UCLA, University of California at Los Angeles, Los Angeles, California, USA

Although the brain was traditionally considered as ‘immunologically privileged’, recent findings have implied an involvement of immune mechanisms in neurological disease and illness, including central nervous system (CNS) malignancies. In this review, we initially focus on aspects of the immune system critical for effective antitumor immunity, as an understanding of normal immunological functions and how they relate to tumor immunology will set a foundation for understanding the unique challenges facing the integration of neuro-oncology and neuroimmunology. We summarize current knowledge of immune responses in the ‘immunologically quiescent’ brain and its role in tumor immunology. We will then discuss the emerging field of ‘immunomics’ and recent advances in molecular technologies, such as DNA microarray, which are being applied to brain tumor antigen epitope discovery and patient stratification for brain cancer immunotherapy. This, in turn, should have significant importance for ultimately designing and developing efficient and focused strategies for anticancer immunotherapy. Finally, the current state of immune-based treatment paradigms and future directions will be discussed, paying particular attention to targeted antibody strategies, adoptive cellular immunotherapy, and tumor vaccine approaches that have been studied in clinical trials for CNS neoplasms.


Keywords: Brain tumor; cancer vaccines; immunomics; immunotherapy; microarray

INTRODUCTION

Tumors arising within the central nervous system (CNS) present the immune system with challenging targets to recognize and eradicate. Anatomical constraints and distinctive immunoreactivity provide a complex microenvironment within the CNS. Tumor heterogeneity and immune defects seen in tumor-bearing hosts add to this complexity, comprising significant impediments for immune based therapies. The relative absence of immune reactivity in the brain and the dismal prognosis of patients with glioblastomas has often been ascribed to the ‘immune privilege’ of the CNS. However, our current understanding of neuroimmunology now reveals that immune responses can and do occur within the CNS and brain tumors (Figure 1). Immunotherapy for cancer represents a particularly appealing form of treatment because of its potential for highly specific antitumor cytotoxicity.

This review will initially focus on aspects of the immune system critical for effective antitumor immunity. An understanding of normal immunological functions and how they relate to tumor immunology will set a foundation for appreciating the unique challenges that brain tumor researchers currently face. We will then discuss the emerging field of ‘immunomics’ and recent advances in molecular technologies that are being applied to brain tumor antigen discovery and patient stratification for immunotherapy. This, in turn, should have significant importance for ultimately designing immunotherapeutic strategies in the future.

INNATE AND ADAPTIVE IMMUNE RESPONSES

Innate immunity

The innate immune response against neoplastic transformation is comprised of natural killer (NK) cells, macrophages (microglial cells in the CNS) and granulocytes. The innate immune response is thought to be critical for stunting the initial burst of tumor cell proliferation during the period of 4–7 days needed to generate an adaptive immune response. Directed against a broad range of targets, the innate immune system detects antigens known as pathogen-associated molecular patterns (PAMP). PAMP are conserved bacterial, viral, parasitic or tumor-derived molecules that are associated with some form of cellular stress. Some examples of PAMP include lipopolysaccharides (Gram-negative bacteria), lipoteichoic acid or mannose.
(Gram-positive bacteria), mannan (fungi), viral envelopes, and heat shock proteins (in neoplastic cells). Primary transmembrane proteins, known as toll-like receptors, are expressed on the surfaces of immune cells and are critical for PAMP detection. The origin of the name ‘toll-like receptors’ (TLR) came from the family of Drosophila proteins called ‘Toll’, which were initially discovered as developmental genes in flies. Toll was later found to have antimicrobial immune functions by protecting Drosophila against fungal infections. Around the same time, it was shown that a similar protein of immunologic significance in mammals also signaled by way of toll-related mechanisms. Hence, the name ‘toll-like receptors’ was used to refer to the mammalian homologs of Toll. TLR can be found on tissue dendritic cells (DC), macrophages, monocytes (microglia in CNS), granulocytes (neutrophils, eosinophils and basophils) and natural killer cells. Upon binding of PAMP, TLR activate cells and elicit potent pro-inflammatory responses (e.g. cytokine release), activating other immune cells that are ultimately responsible for antigen destruction and removal. For example, a bacterium-related antigen may bind to TLR and cause the secretion of factors that induce lysis and phagocytosis. Both of these are accompanied by the release of IL-1, IL-6, IL-12, tumor necrosis factor-alpha (TNF-α), oxygen radicals, nitric oxide, prostaglandins and leukotrienes. TLR recognition of virally derived antigens, however, produces factors that induce cellular apoptosis and the release of type 1 interferons. In either circumstance, these secreted factors also upregulate DC activity by enhancing their migration to the lymphoid tissue and increasing major histocompatibility complex (MHC) presentation, as well as activating a Th1 response. In effect, the TLR-induced innate immune response serves to facilitate the efficient priming of the adaptive immune response.

Adaptive immune response

The adaptive immune response consists of two subtypes, the humoral and cellular immune responses. Both types are highly specific reactions consisting of four stages: recognition/activation, proliferation, effector and memory. While both the humoral and cellular immune responses may act cooperatively and simultaneously during adaptive immunity, each one is suited for the removal of particular forms of antigen.

Humoral immune response

The primary components of the humoral immune response are B cells and antibody-secreting plasma cells (Figure 2). CD4+ helper T cells often assist with the humoral response. Naïve CD4+ Th cells (Th0) may differentiate into either CD4+ Th1 cells or CD4+ Th2 cells. The primary role of both CD4+ subclasses is the release of cytokines that regulate the function of other immune cells. Th2 cells secrete the B cell-stimulating cytokines IL-4 and IL-10 after antigen-presenting cell (APC) interaction. Upon recognition of their specific antigen by B cell receptors on the cell surface, stimulated B cells will differentiate into antibody-secreting plasma cells. Antibodies can then eliminate antigen via complement-dependent lysis, antibody-dependent cell mediated cytotoxicity and/or opsonization of antigen-coated particles. Antibodies detect free and native antigen, making them effective against bacteria or other foreign antigens. Because they lack the ability to cross the blood–brain barrier (BBB) and enter the brain parenchyma, antibodies are not usually efficient eliminators of tumor antigens in the CNS unless they are adaptively transferred directly into the brain.

A subpopulation of activated, antigen-specific B lymphocytes also differentiate into long-lived memory cells. These memory B cells rapidly differentiate into antibody-secreting plasma cells when antigen is
encountered later. The humoral response is critical for defense against the majority of extracellular pathogens, but does not provide effective immunity to intracellular pathogens within tumor cells.

**Cellular immune response**

The cellular immune response exerts its effects primarily through CD4⁺ Th1 helper T cells and CD8⁺ cytotoxic T lymphocytes (CTL) (Figure 3). Unlike its humoral counterpart, the cellular immune response is adapted for the elimination of antigens derived from the cytoplasm of cells. Activated CD8⁺ CTL precursors differentiate and gain the ability to directly lyse tumor or infected cells.

Each form of T cell (CTL versus Th) can only recognize antigens in a particular format. This format is created by the MHC, which has two distinct classes—class I and class II. In terms of presenting antigen, the vast majority of nucleated cells express MHC class I molecules. CD8⁺ T cells are ‘restricted’ to the recognition of antigens in the context of MHC class I, which is conferred by the differences in the expressed form of the T cell receptor. Also known as the direct pathway, MHC class I carries peptides of 8–12 amino acids in length. These peptides are derived from endogenous antigens such as cytoplasmic tumor proteins and viral pathogens. Th cells, however, are restricted to antigens in the context of MHC class II, which are normally related to exogenous antigens and are 12–20 amino acids in length. MHC class II complexes are commonly found on APC or macrophage cell surfaces; and this form of recognition is known as the indirect pathway, because the effector arm acts indirectly through the immune regulation caused by the Th cells.

Naïve T cells of both types continuously circulate through the blood and lymphatics, sampling all antigens within the environment through interactions with APC. Derived from hematopoietic progenitors, DC are professional APC situated at sites of interface with the environment. In the absence of inflammatory stimuli, they continuously sample both self and non-self. In the presence of pro-inflammatory signals or ‘danger signals’, however, DC become activated and increase the uptake of antigens, which are loaded in the context of MHC. Commonly known danger signals include lipopolysaccharides (LPS), viral dsRNA, CpG oligonucleotides, mechanical stress and inflammatory cytokines such as IL-1, IL-2 and TNF-α. Danger signals may lower the threshold for overcoming peripheral tolerance in self antigen-specific T cells, as well as increase DC trafficking, survival and cross presentation. Mature DC lower antigen uptake and upregulate the adhesion and co-stimulatory molecules. After maturation, DC migrate to the paracortical regions of the lymph nodes, where they can encounter naïve CD8⁺ and CD4⁺ T cells.
Here, activation of DC induces cross presentation, increasing MHC expression and trafficking to T cell areas of lymphoid tissue, where DC can initiate effector T cell responses.

The ‘danger signal’ mechanism is not the only safeguard that the immune system has against harmful autoimmunity. Because a breakdown in self-tolerance can be lethal, especially within the CNS, several other mechanisms exist to preserve peripheral tolerance. For instance, only the concomitant stimulation of at least two receptor types on the T cell will drive differentiation into effector cells (Figure 4). The first signal arises when antigens, in the context of the appropriate MHC, bind T cell receptors (TCR) on the surfaces of CD4⁺ and CD8⁺ cells. The second signal is derived from ligands on the APC (i.e. B7 for CTL and CD40 for Th cells). Binding of B7 by receptors on the surface of naïve T cells (e.g. CD28 for CD8⁺ T cells and CD40 for CD4⁺ T cells) with concomitant TCR activation results in cytokine release, clonal proliferation and subsequent differentiation to cytotoxic T cells. These differentiated CTL are then ready to exert their effects as the effector arm of the cellular immune response. In the absence of co-stimulation, however, T cells can develop tolerance to the specific antigen bound to the T cell receptor, and the T cell can become unresponsive or ‘anergic’ to that antigen.

To further guard against deleterious autoimmunity, a unique class of T cells, known as regulatory T cells (T_{reg}), also help maintain peripheral self-tolerance. T_{reg} are distinctive in that they express high levels of negative co-stimulatory molecules, which attenuate activation of CTL. For example, T_{reg} express cytotoxic T-lymphocyte antigen 4 (CTLA-4), which has a higher affinity for CD28 than does B7, but does not co-stimulate T cell activation upon binding. This disrupts normal, positive co-stimulation and increases T cell anergy. T_{reg} also constitutively express CD25 and express high levels of FoxP3, which acts as a transcription factor that is thought to direct the cellular machinery responsible for the tolerance mechanism.

**TUMOR IMMUNOLOGY AND THE CENTRAL NERVOUS SYSTEM**

Once tolerance has been overcome and the effector arm activated, the immune system should theoretically eliminate tumor antigens. In the CNS, however, special environmental circumstances may hinder the actions of the typical progression of antitumor immune responses (Table 1). Although there are currently several examples of successful immunotherapeutic approaches for tumors outside the CNS, the unique properties of the brain have made the direct application of basic immunologic principles more challenging for tumor rejection within the CNS. Such unique immunological properties of the brain initially led researchers to refer to the CNS as ‘immunologically privileged’. However, it is now widely accepted that the CNS should be thought of more as an ‘immunologically quiescent’ site that is unable to initiate immune responses efficiently, rather than a site that is completely devoid of effector immune responses altogether.
The effective initiation of an antitumor immune response requires tumor antigens to be presented to immune cells. Tumors that arise within the confines of the CNS may have anatomical and biological constraints that limit such antigen presentation. Such limitations include the BBB, which, through tight junctions and glial cell barriers, regulates the inflow of immunological materials to a great extent. This may allow insufficient access of immune cells to tumors located in CNS, therefore preventing interactions of systemically activated immune cells with brain tumor cells. The initiation of an antitumor immune response may be further hindered by a lack of recognized tumor rejection antigens, lack of MHC expression and lack of TAP (transporter associated with antigen processing) molecules and professional APC within the brain. Furthermore, even if an antitumor immune response is initiated, the absence of an organized lymphatic system required for immune cell traffic may prevent the infiltration of immune cells into CNS tumors.

Table 1: Possible mechanisms of immune escape by tumors in the CNS

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor antigens are not presented to immune cells</td>
<td>Lack of tumor antigen(s) on tumor cells&lt;br&gt;Lack of MHC class I or II molecules in brain parenchyma&lt;br&gt;Lack of TAP (transporter associated with antigen processing) molecules&lt;br&gt;Lack of professional APC in local CNS tumor environment</td>
</tr>
<tr>
<td>Tumor antigens are presented, but do not elicit an immune response</td>
<td>Tumor peptide density below the threshold level required for T cell activation&lt;br&gt;Oncofetal antigen with established immune tolerance&lt;br&gt;Co-stimulatory signal absent or with inhibitory action (e.g. T_{reg})&lt;br&gt;Appearance of new epitopes that induce tolerance instead of immunogenicity</td>
</tr>
<tr>
<td>Lack of efficacy of systemic immune response</td>
<td>Insufficient access of immune cells to CNS</td>
</tr>
<tr>
<td>Tumor microenvironment suppresses immune responses</td>
<td>Soluble factors (TGF-β2, IL-10, PGE2, etc.)&lt;br&gt;Cell–cell contact (Fas/FasL, galectin)</td>
</tr>
</tbody>
</table>
within the CNS may hinder further immune activity downstream of initiation.

In addition to the constraints imposed by the anatomical segregation of the CNS itself, malignant brain tumors may also induce defects in adaptive antitumor immune responses. Thus, although the CNS can demonstrate the presence of effector immune responses, there are special mechanisms that work antagonistically to hamper the efficacy of such responses. These include immunosuppressive soluble factors (e.g. TGF-β, IL-10 or PGE2) or cell–cell interactions (e.g. Fas/FasL, galectin-1) that lead to T cell death. There is evidence that profound local and systemic immune defects can be observed in patients with CNS malignancies, which may further exacerbate brain tumorigenesis and progression.

In spite of these numerous obstacles, there is recent evidence that the immune system can overcome the immunoreactivity limitations of the CNS during illness and disease (i.e. multiple sclerosis, encephalitis, tumor and transplantion). The exact mechanisms by which this change in immunoreactivity occurs are yet unknown. However, the possibility that the immune system can mediate interactions with the CNS during disease, such as in the development of brain tumors, validates further investigation of immunotherapeutic modes of treatment for brain neoplasms.

### BRAIN TUMOR ‘IMMUNOMICS’ AND REVERSE IMMUNOLOGY

To maximize the utility of brain tumor immunotherapy, however, the identification of relevant tumor rejection antigens is needed. The unveiling of the human genome sequence, along with rapid advances in genomic/proteomic expression profiling, bioinformatics tools, and immunological assay methods, have now made it possible to screen large numbers of tumor-associated proteins for immunogenic epitopes. These technological advances in “immunomics” will likely lead to the design of more rational and specific strategies for CNS immunotherapy in the future.

#### Microarray technology and tumor-associated antigens (‘reverse immunology’)

Despite immune escape mechanisms elaborated by brain neoplasms (Table 1), the concept of effective anti-glioma immune responses has been bolstered by the recent identification of several tumor-associated antigens (TAA) expressed by human brain tumors (Table 2). These include a mutated epidermal growth factor receptor variant (EGFRvIII) mutant p53, p16, AIM-2, SART2, IL-13Rα2, survivin, hTERT, and melanoma antigen-encoding genes (MAGE-1, MAGE-3, gp100, TRP-1 and TRP-2). Screening peptide and cDNA libraries for T cell reactivity can be conducive to identifying additional tumor-related antigens for further study.

With the advent of microarray chip technology that can scan tumors in a high throughput manner for tumor-specific gene expression, more potential glioma-specific antigens will probably be discovered and investigated in the near future. Furthermore, recent advances in our understanding of tumor immunology can link the information generated from gene-expression profiling of CNS tumors to the identification of precise immunogenic epitopes that can serve as useful targets for specific immunotherapy. For instance, since T cells can only recognize antigens in the context of MHC, tumor-associated genes can be scrutinized for amino acid sequences that can interact with relevant MHC molecules. Candidate tumor-specific peptide epitopes for CTLs can now be identified using available computer algorithms that predict the MHC binding affinities of specific peptide and epitope sequences.

<table>
<thead>
<tr>
<th>Category of antigen</th>
<th>Tumor antigen</th>
<th>HLA restriction</th>
<th>Documented CD8+ T cell response to CNS tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique mutant</td>
<td>EGFRvIII</td>
<td>HLA-A2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td>Multiple HLA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>p97</td>
<td>Unknown</td>
<td>–</td>
</tr>
<tr>
<td>Cancer/testis</td>
<td>MAGE</td>
<td>Multiple HLAs</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>GAGE</td>
<td>HLA-A29, Cw6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IL-13α2</td>
<td>HLA-A2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SSX</td>
<td>HLA-A2</td>
<td>–</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Tyrosinase</td>
<td>HLA-A1, A2, A24, B44</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TRP-1</td>
<td>HLA-A31</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>TRP-2</td>
<td>HLA-A2, A31, A33, Cw8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>gp100</td>
<td>HLA-A2, A3, A24, Cw8</td>
<td>–</td>
</tr>
<tr>
<td>Normal proteins</td>
<td>Wild-type EGFR</td>
<td>HLA-A2</td>
<td>–</td>
</tr>
<tr>
<td>with selective</td>
<td>Her2/neu</td>
<td>HLA-A2</td>
<td>+</td>
</tr>
<tr>
<td>or over-expression</td>
<td>hTERT</td>
<td>HLA-A2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SART1, SART3</td>
<td>HLA-A24</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Survivin</td>
<td>HLA-A2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>AIM-2</td>
<td>HLA-A1</td>
<td>+</td>
</tr>
</tbody>
</table>

By testing the stability of MHC molecules with candidate tumor-specific peptides, an estimation of the potential immunogenicity of a peptide can be determined in vitro. The immunogenicity of human cancer genes or their predicted CTL peptide epitopes can also be assessed in animal models using HLA transgenic mice, providing even better estimates for immunotherapy targets in vivo. Recently, experiments involving the screening of human tumor samples for CTL reactivity have identified human glioma-associated antigen epitopes specifically recognized by the immune system. Based on these recent advances, it is suggested that cancer genomics can be directly linked to tumor immunotherapy by ‘reverse immunology’, which paves the way for the design of new, more effective treatments.

---

Published by Maney Publishing (c) W. S. Maney & Son Limited
specific targets for brain tumor immunotherapy. This concept of ‘tumor immunomics’ \(^{37}\) (illustrated in Figure 5) has given new momentum to the daunting task of adding characterized TAA to the currently sparse list of effective antigenic targets for brain tumor immunotherapy.

**Microarrays and patient stratification for immunotherapy**

In addition to target antigen discovery, DNA microarray analysis of brain tumor specimens can also be used for molecular classification and patient stratification for immunotherapy. Although numerous immunotherapeutic approaches may hold great promise for the treatment of patients with brain cancer, a major challenge to the realization of clinical efficacy using these treatments is to determine which individual patients are most likely to benefit from the various forms of immunotherapy. DNA microarray-based technologies, which allow simultaneous analysis of the expression of thousands of genes, have already begun to uncover previously unrecognized patient subsets of brain cancer that differ in their survival \(^{31,32,38}\). The fact that DNA microarrays can be used to detect molecular subsets that strongly predict survival indicates that it will soon be possible to develop gene-based predictors of therapeutic response, including responses to specific immunotherapies.\(^{39}\)

One of the most promising applications of microarray technology is class distinction and patient selection for clinical trials (including immunotherapy protocols) through gene expression profiling as a diagnostic, therapeutic and prognostic tool. Recent studies have shown that gene-expression signatures are relatively robust across an individual tumor, regardless of the spatial location of the tumor sample. Thus, despite the spatial heterogeneity in the histological morphology of cancer cells within a tumor, the gene-expression profiles between spatially distinct biopsies of the same tumor are relatively consistent.\(^{40}\) It has also been found that the variation in gene expression is much greater between samples from different patients, despite the same histological diagnosis, than from multiple samplings of a given tumor from an individual patient.\(^{38,40}\) Therefore, the varied immunogenicity of shared tumor antigens among different patients owing to heterogeneous levels of antigen expression and differing levels of immunoreactivity of patients indicates that individualized immunotherapy should ideally be performed.\(^{41}\)

Systematic gene expression analysis by DNA chip/microarray technology will lead to a better understanding of the nature of brain tumor antigens and changes in antigen gene expression before and after immunotherapy. This information will allow for the development and improvement of immunotherapeutic strategies by identifying patients who are most likely to benefit from immunotherapy and those who harbor immunotherapeutic escape mechanisms. Based on this information, more rational hypotheses about the various mechanisms of tumor immune escape and how to overcome the identified problems will allow for the future design of more rational immunotherapeutics for CNS malignancies. With new genetic tools, such as DNA microarray analysis, significant progress in the treatment of neoplasms in the ‘immunologically privileged’ brain should be forthcoming.\(^{42}\)

**METHODS OF TARGETED IMMUNOTHERAPY**

In addition to the identification of immunogenic tumor rejection antigens and the selection of appropriate patients, successful brain cancer immunotherapy also depends on the development of effective methods of delivering antitumor immunity to the CNS. Various types of immunotherapy strategies, both passive and active, have been used to target intracranial neoplasms.

**Passive immunotherapy**

In passive (adoptive) immunotherapy, immunity is exogenously ‘transferred’ by injecting either antibodies or T cells specific for an antigen. Therefore, a host ‘passively’ acquires immunity, thereby not having to effectively generate immunity using endogenous immune cells. This mode of therapy can be advantageous when a patient is severely immunocompromised as a result of their disease.

Serotherapy is a form of passive immunotherapy that involves the administration of antigen-specific antibodies. Tumor-specific antibodies used to target CNS glioma cells and deliver radiochemicals or immunotoxins to the tumor site have been extensively investigated.\(^{33,44}\) Clinical experiences with radiolabeled anti-tenascin mAb 81C6 have resulted in statistically significant increases in survival and prolonged disease stabilization.\(^{45}\) Clinical trials of immunotoxins for CNS neoplasia have been conducted using TNF-CRM107 (human diphtheria transferin + dipheria toxin mutant CRM107), and *Pseudomonas aeruginosa* exotoxin A mutants conjugated to interleukin-4 (IL-4-PE38KDEL), interleukin-13 (IL-13-PE38QQR),\(^{46,47}\) or transforming growth factor-alpha (TP-38)\(^{48}\). These initial trials of passive serotherapy have shown some significant antitumor effects, which have spurred further multicenter phase I/III trials.

Adoptive transfer of lymphokine activated killer (LAK) cells, allogeneic fibroblasts and expanded systemic T cells have also been used to treat malignant glioma patients. Early studies focused on the adoptive transfer of non-antigen-specific LAK cells and rhIL-2 for CNS gliomas.\(^{41}\) Even though sporadic cases of improved survival were reported, the non-specificity of LAK cells and the neurotoxicity associated with high-dose IL-2 hampered further studies. In pre-clinical animal models, prolonged survival was observed in mice treated with semi-allogeneic fibroblasts secreting IL-2\(^{52}\). In recent years, greater tumor specificity was achieved with adoptive immunotherapy using tumor-sensitized or alloreactive CTLs, which have shown improved survival in pre-clinical models and in phase I clinical trials for CNS gliomas.\(^{53,54}\) However, poor tumor-specific trafficking and rapid disappearance of transferred T cells...
cDNA microarrays can identify differentially expressed, overexpressed or deleted genes in glioma.

Genes identified from microarrays can then be tested for their quantitative expression by real-time, quantitative RT-PCR in different tumor samples.

The full DNA and amino acid sequences are obtained from Genbank and scrutinized for potential CTL epitopes using computer algorithms.

Predicted glioma-associated peptides are synthesized and tested for their ability to stabilize HLA molecules.

HLA-restricted CTL epitopes are then tested for their ability to prime glioma-specific T cells in the HLA-A2/Kb transgenic animal model.

Test whether CNS glioma-bearing patients have glioma-associated CTL epitope-specific T cells using soluble peptide/HLA tetramers.

Begin Phase I clinical trial using glioma peptide-pulsed dendritic cells.

Figure 5: Diagram showing the necessary steps to link genomics with tumor immunology for the discovery of novel tumor antigens for CNS malignancies. Genes that are over-expressed in gliomas are identified initially by differential gene expression analysis (using DNA microarray, SAGE, etc.). These genes are analysed for their role in tumorigenesis and/or for their selective expression in brain tumors. Candidate antigens are tested further, using methods of epitope deduction. This includes peptide amino acid prediction, binding to HLA, and T cell repertoire analysis in animal models and human samples. (Figure adapted from ref. 35 by the kind permission of Elsevier Publishers)
still limited the usefulness of this strategy. To improve tumor-specific targeting, a recent study utilizing the adoptive transfer of interleukin-13-zetakine re-directed cytolytic T cells for tumor-specific recognition and killing of glioblastoma cells has shown notable success in xenograft models, and phase I clinical trials in recurrent glioblastoma patients have been initiated to test the feasibility of this treatment modality.59.

**Active immunotherapy**

Active (vaccine) immunotherapy seeks to generate or augment endogenous host immunity by specifically boosting the host’s own immune response to antigen(s). Many studies of cancer vaccines have used cytokine gene therapy approaches to generate antitumor immunity. These pioneering studies were performed over a decade ago and demonstrated that cytokine-secreting tumor cells could induce antitumor immunity.56 With CNS tumor models, cytokine gene therapy studies have been able to demonstrate efficacious antitumor immunity to intracranial gliomas secreting IL-2, IL-4, IL-6, IL-7, IL-12, GM-CSF, mM-CSF and IFN-α/β.57–59 These studies reveal the complexity of the multiple mechanisms by which antitumor immunity can be generated.

DC-based vaccine mechanisms are yet another form of active immunotherapy. As mentioned above, inflammatory signals that are elaborated by professional APC, such as DC, can subsequently prime potent tumor-specific T cell responses. Thus, investigators have sought to enhance tumor-specific antigen presentation to T cells using ex vivo generated DC. This therapy exploits the ability of DC to prime T cells to tumor-derived antigens. DC are generated ex vivo and loaded with any of a wide array of tumor-derived antigens, and are then re-infused into the host to augment the immune response. Preclinical animal studies have demonstrated that DC pulsed with tumor peptides, RNA and/or tumor lysates can elicit antitumor immune response against CNS neoplasms in vivo.60–63. To date, the safety and feasibility of DC therapy has been supported by recent data from several phase I clinical trials and a multicenter phase II clinical trial of DC vaccination for patients with newly diagnosed glioblastoma is underway. Although the clinical data to date offers too limited information to make any conclusions about efficacy, the potential advantages of DC-based immunotherapy, along with its safety and feasibility, make this a promising immunotherapeutic option. Despite such promise, however, there are still some practical and theoretical problems for the clinical development of DC-based vaccines, as several variables are at play within the context of therapy modulation. Examples include the source of the antigens used for priming (e.g. tumor lysates, specific peptide epitopes, or RNA), origin of the DC used (e.g. spleen or bone marrow-derived peripheral blood mononuclear cells), and DC maturation status (e.g. immature versus mature).59,70 In addition, dose, frequency and route of injection are also important considerations of the effectiveness of the vaccine and require careful manipulation. Furthermore, adjuvants to DC vaccines have also shown promising preliminary data. By exploiting the mechanisms of the danger hypothesis (described above), adjuvants such as LPS, Poly IC:LC (Ampligen RTM), CpG, and the toll-like receptor-7 (TLR-7) agonist imiquimod (Aldara®) can potentiate DC activation, strengthen antigen presentation, and even enhance therapeutic efficacy.

Another modality by which to deliver tumor antigens to the immune system involves the use of attenuated, recombinant viruses and/or bacteria. Viral/bacterial infections and the resulting tissue damage can also provide the appropriate ‘danger signals’ to attract professional APC necessary for adequate antigen presentation. Using recombinant techniques, tumor antigens have been introduced into viral and bacterial vectors (e.g. Vaccinia, Listeria) to improve APC recognition of the tumor epitopes and subsequent APC antigen presentation to CTL.64–66. Direct infection of APC by viruses/bacteria expressing tumor antigens could result in endogenous processing of tumor epitopes in the context of MHC class I, as well as antigen presentation via MHC class II pathways. This improved antigen processing/presentation, along with the danger signals associated with infection, may break tumor tolerance and enhance specific antitumor immunity within the CNS.

Furthermore, vaccine strategies that stimulate the presentation of tumor-specific antigens to T lymphocytes may be particularly beneficial for CNS tumors because they have been found to induce determinant spreading, which is a phenomenon whereby T cells can recognize shared endogenous glioma epitopes along with the targeted antigen(s).76. Given the heterogeneity of human brain tumors and the possibility of immune escape of tumor cells, exploiting the process of epitope spreading will obviously be valuable in the clinical context of designing tumor vaccines in the future.

**CONCLUSION AND FUTURE DIRECTIONS**

The virtual absence of significantly improved survival for patients with CNS tumors in the last decades indicates that novel strategies for treating these neoplasms must be explored intensively. Experimental immunotherapeutic strategies represent attractive complements to treatments based on surgery, radiation and traditional chemotherapy, all of which have proved rather disappointing. The impressive advances that have been achieved in the understanding of tumor immunology and tumor genomics/immunomics provide novel ways to attack brain cancer.

Because of encouraging results in many phase I studies, phase II clinical trials of immunotherapy should be pursued. However, it should be noted that, as with any other targeted treatment modality for brain tumors, immunotherapy trials might only realize significant potential clinical efficacy if given to the appropriate subgroup of patients and/or if administered in combination with other therapies. With the current experience in cancer treatments, it appears that simultaneously...
targeting several components essential to the neoplastic process should provide maximal chances of tumor control. Therefore, therapies based on immunoenhancement and cancer vaccines could be combined with the traditional surgery, radiation and chemotherapy. Such integrated treatment strategies may prove to be of low toxicity and should be synergistic. In addition, the combined use of conventional treatments within the context of clinical trials of immunotherapy will allow evaluation of efficacy, yet retain the ethical requirements for human investigation.

ACKNOWLEDGEMENTS

We are grateful to the generous support of the Kenneth A. Jonsson and Phillip R. Jonsson Foundations, the Neidorf Family Foundation and the Musella Foundation for Brain Tumor Research.

REFERENCES

Central nervous system malignancies: Haumith R. Khan-Farooqi et al.


76 Heimberger AB, Crotty LE, Archer GE, et al. Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity

702 Neurological Research, 2005, Volume 27, October