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CELLULAR IMMUNITY AND IMMUNOTHERAPY OF BRAIN TUMORS

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1. ABSTRACT

Factors influencing the host immune response to central nervous system (CNS) tumors are not yet well understood. This review will outline what is known about anti-tumor immune responses against CNS tumors and describe how advances in our knowledge of basic immunology may be applied to brain tumor immunotherapy. We will first focus on cellular immune system interactions involved in peripheral anti-tumor immune responses. Then, we will discuss characteristics of tumors arising within the confines of the CNS that distinguish them from peripheral neoplasms, emphasizing immune defects that seem to limit or curtail specific anti-tumor immunity against brain tumors. Finally, the current state of immune-based treatment paradigms and future directions will be discussed, paying particular attention to adoptive cellular immunotherapy and tumor vaccine approaches for the treatment of malignant gliomas.

2. INTRODUCTION

Tumors arising within the central nervous system (CNS) present the immune system with a difficult target to recognize and eradicate. The anatomical constraints and distinctive immunoreactivity imposed by the CNS, coupled with the extensive immune defects seen in tumor-bearing animals and patients, comprise significant impediments for the immune system. Gliomas invade normal brain parenchyma and infiltrate along nerve tracts and Virchow-Robbins spaces, as well as migrate within the brain to sites distant from the primary malignancy. Despite recent advances in traditional treatment options, the prognosis for these patients has not changed appreciably. The five-year survival rate for patients harboring the most common class of gliomas, glioblastoma multiforme (GBM), is less than two percent (1), with a median survival of less than two years despite aggressive therapy. In an effort to improve the outcome of patients with brain cancer, there have been attempts to give adjunctive therapies consisting of radiation with or without chemotherapy. Thus far, research over three decades has failed to provide definitive evidence of improved outcome (i.e., overall survival, disease-free survival) in GBM patients.

The successful rejection of syngeneic tumors, whether they are growing at peripheral sites or within the CNS, is usually dependent upon T lymphocytes and their secreted products (2). However, there is a complex interplay between immune cells of the innate immune system and the adaptive immune system that influence the ability to mount an effective anti-tumor immune response. The relative absence of immune reactivity in the CNS and dismal prognosis for patients with CNS gliomas has often been ascribed to the "immune privilege" of sites such as the brain and anterior chamber of the eye (3). However, our current understanding of neuro-immunology now reveals that cellular immune responses can and do occur within the CNS. It is now well accepted that the CNS can activate the innate and adaptive immune systems, for example by expressing Toll-like receptors (TLRs) and complement components, or using microglia as antigen-presenting cells (APC). The CNS should be thought of more as an "immunologically quiescent" site -- a well-balanced environment with a predominant expression of immunosuppressive mediators that delay or hamper immune responses (4,5).

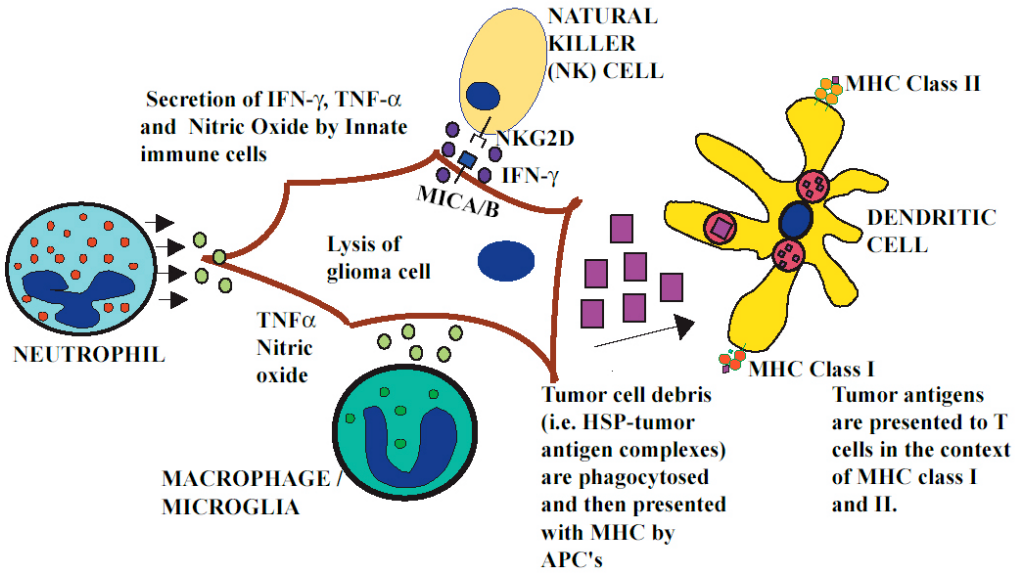

Figure 1. Innate Anti-tumor Immune Response. Schematic summarizing the innate immune response to gliomas. This "first wave" response involving tissue macrophages/microglia, neutrophils, natural killer (NK) cells, and dendritic cells (DC) may be critical for the subsequent adaptive tumor-specific response.

3. INTERACTIONS BETWEEN THE INNATE AND ADAPTIVE IMMUNE SYSTEMS THAT INDUCE ANTI-TUMOR IMMUNITY

3.1. Innate Immune System Interactions

The innate immune response consists primarily of leukocytes that respond quickly and remotely to *non-specific* signals characteristic of tissue damage and infection. Innate immune cells such as dendritic cells (DC), tissue macrophages and monocytes (microglia in the CNS), granulocytes (neutrophils, eosinophils, basophils), and natural killer (NK) cells are able to sense or detect conserved pathogen-associated molecular patterns (PAMP) or proteins associated with cellular stress/transformation (6-8). Macrophages, dendritic cells, and microglia express many members of an expanding family of TLRs that respond to distinct PAMPs (5). Specifically for tumor development, it has also been demonstrated that tumor cells up-regulate proteins such as MHC class I chain related (MIC), retanoic acid early inducible (Rae-1), H-60, and UL16 binding proteins (ULBP). These stress-induced proteins can interact with a specific family of NK receptors (NKG2D) expressed by NK cells, macrophages and $CD8^+$ T cells (6,9). NKG2D has been found to be expressed differently in mice and humans. Murine NKG2D is expressed on all NK cells, a subset of γδ T-cells, macrophages, and activated $CD8^+$ T cells; while humans do not express NKG2D in macrophages, but express this receptor in $\alpha\beta TCR^+/CD8^+$ T cells regardless of their activation status (9). The recognition of inducible tumor-associated proteins and TLR by innate immune cells elicits cytotoxicity and IFN-γ secretion by NK cells, along with release of nitric oxide (NO) and TNF-α by macrophages (6). Friese *et al* have recently published data on the expression profile of NKG2D ligands on experimental gliomas (10). Indeed, the ectopic over-expression of NKG2D ligands on glioma cells renders these cells susceptible to T-cell and NK-cell targeting, both *in vitro* and *in vivo* (10).

Tumor-associated proteins do not directly need to interact with innate immune cell receptors, though. It has also been observed that heat shock proteins (HSP), and their bound peptides from the cytoplasm, are liberated from necrotic tumor cells and efficiently activate macrophages and dendritc cells (11-13). The specificity of the anti-tumor immune response is derived from the tumor-associated peptides bound to HSP, which are endocytosed via the α-macroglobulin receptor. Dendritic cells and other antigen presenting cells (APC) endocytose these HSP-peptide complexes, resulting in the transport of the tumor-associated peptide on the cell surface together with MHC class I molecules (11). The activation of DC by HSP may derive from the ability of HSP to interact with the toll-like receptor 2/4 pathway (14,15). CNS tumors, including high-grade astrocytomas and metastatic tumors, have been shown to express a variety of HSP (16-18). A definitive association between the expression of these HSP by CNS tumors and an immune response has not been established, but could theoretically provide an efficient therapeutic target when pulsed with dendritic cells *ex vivo* (19,20).

Together, these tumor-associated stimuli can efficiently trigger the innate immune response (Figure 1), which may be critical for the activation of the adaptive tumor-specific immune response, thereby facilitating the priming, effector functions, and immune memory of T cells against brain tumors.

3.2. Priming of Adaptive Immune Responses for Anti-tumor Immunity

The presentation of tumor-associated peptides by DC can activate both helper T lymphocyte (T_H) and cytotoxic T lymphocyte (CTL) responses. $CD8^+$ T cells are

often called CTL because they can acquire cytolytic potential to lyse infected or neoplastic cells. $CD4^+$ T cells have historically been called helper T cells because they typically secrete a wide variety of cytokines that enhance the function of other immune cells. More recently, however, it has been discovered that not all $CD4^+$ cells are helpers. There are important populations of $CD4^+/CD25^+$ T regulatory cells (T_{Reg}) that can mediate immunological tolerance (21), while some $CD4^+$ cells even possess lytic function against MHC class II-positive tumor targets. Unlike $CD8^+$ CTL precursors, which can only differentiate into effector CTL, naïve $CD4^+$ T cells can differentiate into distinct types of effector cells depending upon the nature of the stimulus. Naïve T cells (T cells that have not previously seen antigen) continuously circulate through the lymphatics and bloodstream until they encounter APC presenting peptides that they recognize. After recognition of a specific foreign antigen in the proper conformation with an APC, T cells undergo a series of changes in which they secrete cytokines and undergo clonal proliferation with subsequent differentiation into effector T cells.

The ability of DC to prime T cells to tumor antigens within the CNS, however, is a controversial issue because little is known about where this priming normally occurs and the type of APC responsible. As Walker *et al* illustrated in a recent review on the topic, the priming of T cells to brain tumor-derived antigens can understandably occur in three hypothetical ways: 1) DC are recruited to the CNS tumor site by inflammatory stimuli where they phagocytose antigenic material and traffic to the draining lymphoid tissue to activate naïve, brain tumor-specific T cells; 2) Tumor cell debris and antigenic particles drain from the brain to secondary lymphoid tissue, whereupon antigen can be phagocytosed and presented to naïve T cells; or 3) CNS tumor cell material is phagocytosed by parenchymal APC (e.g., microglia), followed by migration to the draining lymphoid tissue. Regardless of the process by which naïve T cells are primed against CNS tumor antigens, many critical steps are not yet understood. Future experimental studies will not only need to define the cell types involved, but also the immune interactions within the brain that determine activation versus tolerance to CNS tumor antigens. These issues are likely to be important because it has recently been reported that DC isolated from brain tissue show an immature phenotype and may contribute to an inhibition of T-cell priming (22).

3.3. Anti-tumor Immune Effector Functions within the CNS

Early studies by Hickey *et al* were some of the first to document that activated T cells could cross the blood brain barrier (BBB) to enter the brain parenchyma (23). More recently, others have shown that tumor-sensitized T cells can also infiltrate intracranial tumors and induce anti-tumor immunity (24-26). The functional requirements of T cells infiltrating CNS tumors have not, to date, been examined in detail. Mukai *et al* demonstrated that the anti-tumor immunity induced by adoptive transfer of tumor-sensitized T cells in a subcutaneous tumor model was contingent upon the transferred T cells infiltrating the tumor (27), but these same requirements have not been examined for CNS tumors. Once T cells have crossed the BBB and entered the brain parenchyma near or within a CNS tumor, the next question is whether these cells still require further activation to acquire their full effector functions or whether simply the recognition of cognate antigen complexed within MHC class I or II is sufficient for anti-tumor immune responses. The complete answer to this question is not known yet. However, other studies by Mukai *et al* have documented critical cellular interactions between tumor-infiltrating T cells and macrophage-type cells found in and around CNS tumors that are necessary for T-cell effector functions at the tumor site (28). More recently, Calzascia *et al* described how interactions of host APC found within CNS gliomas are involved in both the retention of tumor-specific $CD8^+$ T cells within brain tumors and the cross-presentation of tumor-associated antigens (29). These studies have collectively demonstrated that tumor-associated T cells infiltrating CNS tumors interact with host APC at the site, which likely influences the activation state and effector functions of these T cells.

4. IMMUNE DEFECTS TO OVERCOME FOR ANTI-TUMOR IMMUNITY IN THE CNS

4.1. Anatomical and Micro-environmental Factors within the CNS that Limit Immune Responsiveness

Tumors that arise within the confines of the CNS do not seem to induce the exact same type and extent of immune responses as those found peripherally. Certain hallmarks of the CNS can explain why immune responses that occur within the brain do not behave like responses at other peripherally located anatomic sites. Properties of CNS tumors that contribute to this limited immune reactivity include anatomical constraints (i.e., blood brain barrier, lack of traditional lymphatic drainage), high concentrations of immunoregulatory factors (i.e., TGF-β, PGE2, IL-10, gangliosides), and altered MHC expression on parenchymal cells within the CNS for T lymphocytes to interact with (30,31). These inherent properties of the CNS do not, however, explain the full extent of immune defects observed in patients harboring malignant gliomas. Defects in antigen presentation and T lymphocyte function have also been consistently observed in these patients and are outlined below.

4.2. Defects in Adaptive Anti-Tumor Immune Responses Against CNS Tumors

In addition to the constraints imposed by the anatomical segregation of the CNS itself, malignant brain tumors also induce local and systemic immune defects. The immune defects have been linked to tumor-derived soluble factors and/or T cell-tumor cell interactions. For example, cytokines such as TGF-β and IL-10 are often secreted by gliomas and can inhibit the anti-tumor effector functions of T cells (32,33). The alteration of MHC class I expression by glioma cells has also been recognized as a way that tumor cells can influence antigen-specific T-cell and NK-cell recognition. For example, $CD8^+$ T cells can only recognize their cognate antigen when complexed within MHC class I, and thus the hypothetical down-regulation of MHC class I expression in glioma cells could

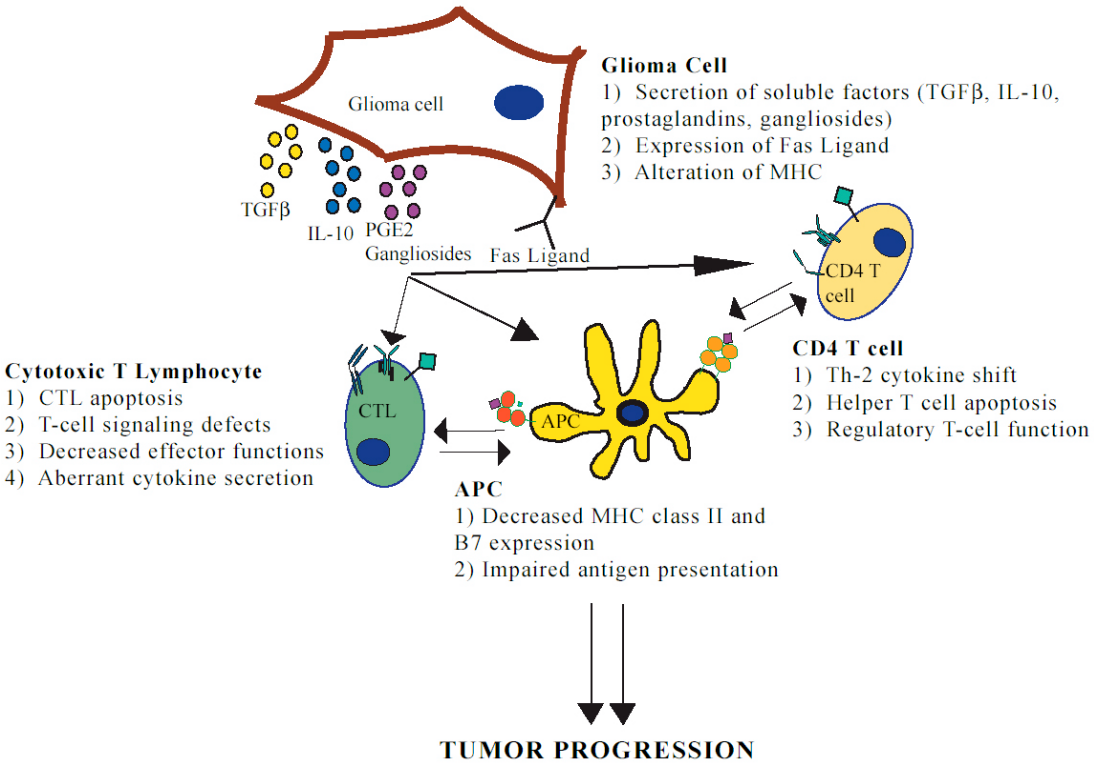

Figure 2. Mechanisms of Immune Escape by Glioma. Schema showing possible escape mechanisms elaborated by glioma cells to evade the immune system. Although some are hypothetical, these include the secretion of soluble factors, expression of Fas ligand, and downregulation of MHC. Such mechanisms, in turn, may cause functional defects in antigen-presenting cells (APC), helper T cells (T_H), and cytotoxic T lymphocytes (CTL).

theoretically prevent $CD8^+$ T-cell lysis of tumor cells. However, receptors on NK cells sense MHC down-regulation, prompting NK lysis of MHC-deficient tumor cells. Therefore, a balance seems to exist between tumor cell capacity to evade T-cell versus NK-cell recognition by modulating MHC class I expression. Today, it is still controversial whether there is actually a downregulation or upregulation of MHC class I expression in glioma cells (34).

APC function can also be influenced in tumor-bearing hosts. Progressively growing tumors can secrete soluble factors or otherwise negatively influence APC maturation and function (35-37), which can impair the ability of APC to prime tumor-specific T cells or impair active immunotherapy approaches. T cells from tumor-bearing animals and patients have been shown to exhibit defects in the expression of multiple signaling molecules involved in T-cell activation (38-44), as well as an increased propensity to undergo apoptosis (45,46). Even though T cells can be found within brain tumors, these tumor-infiltrating T cells may fail to provide effective anti-tumor immunity. The mechanisms behind the inability of tumor infiltrating lymphocytes to provide an effective immune response are not completely understood. However, recent studies from subcutaneous tumor models have suggested that tumor-specific T cells may have been rendered functionally defective or anergic early in tumor progression (47,48) or harbor defects in the critical molecules involved in the lysis of tumor cells when interacting with tumor cells at the site (49,50). Other studies have shown that tumor infiltrating lymphocytes within murine tumors may not receive adequate survival signals to acquire their lytic potential, thus acquiring an immunosuppressive phenotype that impedes anti-tumor immunity (44).

Together, these alterations may allow glioma cells to evade T cell-mediated eradication and lead to tumor progression (Figure 2). This further emphasizes some of the immunological hurdles that cellular anti-tumor immunity must overcome before effective immunotherapy can be generated against brain tumors.

5. NEW AND FUTURE STRATEGIES FOR THE IMMUNOTHERAPY OF CNS TUMORS

5.1. Immunomics and Identification of Tumor-Associated Antigens in Neuro-Oncology

With rapid advances in genomic/proteonomic expression profiling, bioinformatics tools, and immunological assay methods, the identification of tumor rejection antigens recognized by $CD4^+$ and $CD8^+$ T cells has greatly increased the pace of immunotherapy for malignancies such as melanoma (51,52). Specific immunotherapy for malignant brain tumors may also

Table 1. Tumor Rejection Antigens Expressed on CNS Tumors

Tumor Antigen	CNS Tumor Expression [1]	HLA Restriction	Documented $CD8^+$ T cell Response to CNS tumor	Reference
Tyrosinase	G, A, E	HLA-A1, A2, A24, B44	-	61
TRP-1	G, A, E, M	HLA-A31	-	61
TRP-2	G, A, E, M, O	HLA-A2, A31, A33, Cw8	+	61,76
Gp100	G, A, E, M, O	HLA-A2, A3, A24, Cw8	+	120
NY-ESO	N	HLA-A2, A31,	+	121
P97	G,A,E,M,O	Unknown	-	61
SSX	A, O	HLA-A2	-	122
SART	G, A, S	HLA-A24	+	123,124
MAGE	G, A, M, O, MD, E	Multiple HLA's	+	61,122,125,126
GAGE	G, E, MD	HLA-A29, Cw6	-	125
Her2/neu	G, A	HLA-A2	+	120
Survivin	G, A, M, S	HLA-A2	-	127,128
IL13R α2	G	HLA-A2	+	54,77

[1] G, glioblastoma; A, astrocytoma; E, ependymoma; M, meningioma; N, neuroblastoma; S, Schwannoma; MD, medulloblastoma

benefit from such advances in cancer genomics and tumor immunology. Glioma-specific or glioma-associated genes that play a functional role in oncogenesis may provide better and safer immune targets than non-specific inflammatory signals and/or cytokines. A variety of over-expressed and/or differentially expressed genes have been identified from brain tumors using cDNA microarrays, subtractive hybridization, differential immuno-absorption, representational difference analysis, differential display, and other related techniques (53-59). Many of these genes have also been functionally linked to the progression of glioma.

Studies have also shown that genes such as EGFRvIII, IL13R, PTEN, tenascin, melanoma-associated antigens, granulin, SPARC and p53 can be differentially expressed or lost by glioma cells, and even functionally involved in neoplastic transformation (54-55,60-66). Despite their restricted or over-expression in glioma, it is generally unknown whether the protein product of any of these genes can be recognized by the immune system. Glioma-associated antigens such as IL13R, tenascin and EGFRvIII can be recognized by specific monoclonal antibodies for the use in tumor targeting and visualization (54,67-69), but their use may be limited in practice by the inability of circulating antibodies to traffic specifically to sites of microtumor. T cells are ideally suited for this purpose.

However, since T cells can only recognize antigens in the context of MHC, tumor-associated genes must be scrutinized for amino acid sequences that can stabilize relevant MHC molecules. Candidate tumor-specific peptide epitopes for T cells can now be identified using available computer algorithms that predict the MHC binding affinities of peptides (http://bimas.dcrt.nih.gov/molbio/hla_bind/, http://www.uni-tuebingen.de/uni/kxi/). By testing the stability of MHC molecules with candidate tumor-specific peptides, an estimation of potential immunogenicity can be determined (70). The immunogenicity of human cancer genes and/or their predicted peptide CTL epitopes can also be assessed *in vivo* using HLA-A2 transgenic mice (71,72), providing even better estimates of immunotherapeutic targets. New studies are now routinely being reported that are characterizing new antigens expressed on CNS tumors. A listing of antigens expressed on brain tumors that have characterized T-cell epitopes is presented above (Table 1). A more extensive review with a complete listing of all tumor antigens recognized by T cells has also been compiled previously (73).

Recently, glioma-associated antigens recognized by the immune system have been identified (74-77) and shown to protect against intracranial tumor formation in pre-clinical studies (74,75). Based on these recent advances, cancer genomics can be linked to tumor immunology, a concept known as "tumor immunomics" (70), which may point the way to the design of new and more specific targets for brain tumor immunotherapy **(Figure 3).**

5.2. Cellular Adoptive and Active Immunotherapy Strategies for Brain Tumors

Despite the many therapeutic obstacles posed by progressively growing tumors, much has been learned in recent years concerning how the immune system is regulated and can be manipulated to target tumor cells. Immune-based therapies for CNS gliomas have traditionally lagged behind those for other peripheral tumors, but recent advances in molecular genetics and immunology have enabled scientists to begin formulating immunotherapeutic strategies that can, in some instances, protect against experimental CNS tumors and even treat established brain tumors. This section will detail some of the new and exciting modalities for enhancing anti-tumor T-cell immunity using adoptive and active *cellular* immunotherapeutic strategies that might be exploited for CNS gliomas. New serotherapy approaches (i.e., antibody-based adoptive immunotherapy) for brain tumors, which

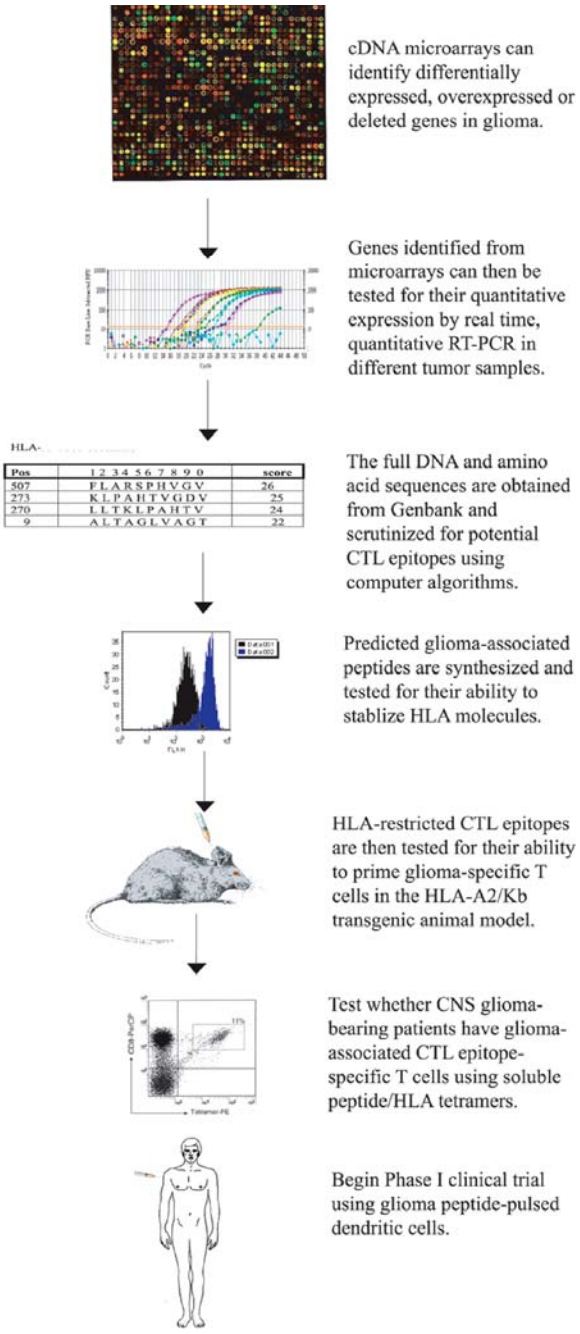

Figure 3. Immunomics in Neuro-Oncology. Schematic diagram elucidating the necessary steps to link genomics with cancer immunology for the discovery of novel tumor antigens for CNS malignancies. Genes that are overexpressed in gliomas are identified initially by differential expression analysis (using cDNA microarray or SAGE). These genes are analyzed for their role in the tumorigenesis and/or for their differential 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 (70) with permission.]

are also being actively investigated with some promising results, have recently been reviewed elsewhere (31) and will not be covered in this review.

T cells are crucial components of cellular immunity and immunotherapy. Because T lymphocytes and their secreted products are critical for the immunosurveillance of tumors (2), and because virtually all signals to T lymphocytes are initiated at the cell membrane, immunotherapies designed to activate antigen-specific T lymphocytes via the TCR/MHC complex and associated co-stimulatory molecules may significantly advance the field. For defined tumor antigens, the design of anchor-modified vaccines and antigens modified to enhance TCR affinity can significantly enhance anti-tumor immune responses (78-82). The issues of peripheral tolerance have yet to be explored for anti-tumor immunity within the CNS, partially because of the paucity of well-defined T-cell epitopes for brain tumors that can be immunologically studied. Nevertheless, strategies designed to overcome T-cell tolerance to tumor-associated antigens are likely to become important in the field of neuro-oncology.

Cellular adoptive immunotherapy approaches have been used in pre-clinical models and human clinical trials for various types of malignancies because the T cells and/or lymphokine activated killer (LAK) cells can be expanded *in vitro* without the immuno-regulatory influences of the progressively growing tumor and then re-infused in large quantities. For gliomas, the adoptive transfer of LAK cells and expanded systemic T cells have been used to treat pre-clinical glioma models (83,84) and malignant glioma patients (85-90). Even though sporadic cases of improved survival were reported, the non-specificity of cell killing and toxicity associated with high-dose IL-2 hampered further studies. Subsequently, adoptive immunotherapy using tumor-sensitized T lymphocytes and/or tumor infiltrating lymphocytes (TIL) has shown some improved survival in pre-clinical models and in a Phase I clinical trial for CNS gliomas (25,26,89,91-93). However, poor tumor-specific trafficking and disappearance of transferred T cells still limited the usefulness of this approach. More recently, the combination of a non-myeloablative chemotherapy regimen before the adoptive transfer of high affinity, tumor-specific T cells in patients with metastatic melanoma has resulted in the persistent clonal repopulation of the transferred cells and regression of metastatic tumor nodules (52). This study highlights the complex homeostatic mechanisms that regulate the number of peripheral T cells and suggests that the ablation of endogenous lymphocytes could enhance the persistence and expansion of clonal populations of tumor-specific, transferred T cells. These new results could reinvigorate the interest for adoptive immunotherapy in glioma, especially when new defined glioma rejection antigens are identified.

By far the most studied immune-based therapies have used active strategies designed to prime or augment the host anti-tumor immune responses. Strategies used in the pre-clinical or clinical treatment of CNS tumors have usually followed studies that targeted other systemic tumor

models. The pioneering studies in peripheral tumor models were performed over a decade ago now and demonstrated that cytokine-secreting tumor cells could induce anti-tumor immunity (94,95). Similar studies have been conducted with CNS tumor models and have had, in general, less success. For example, rat glioma cells (T9, 9L, RG-2) transduced to express IL-2 or IFN-γ could induce an effective anti-tumor immune response when injected at a subcutaneous location, but often caused adverse events and decreased survival when inoculated in the brain (96-99). The decreased survival seen in studies using intracranial (i.c.) delivery of cytokine-secreting gliomas was attributed to complications induced by vasogenic brain edema, but not the absence of an immune response. As such, peripheral (subcutaneous) vaccinations of cytokine gene-transfected tumor cells have been shown to stimulate immunity against tumors located in the "immunologically privileged" CNS, without the problems and excess of inflammatory responses associated with i.c. delivery (100-106). These findings highlight that although immune reactivity within the CNS is different than that in peripheral anatomical sites, the brain is not "immune privileged." These studies also reveal the complexity of the mechanisms by which anti-tumor immunity can be generated.

It is apparent that the early phase of the anti-tumor immune response can sometimes be critical for immunotherapy. This early innate immunity is mediated by an influx of endogenous, non-specific immune cells such as macrophages and neutrophils, which can then promote the subsequent generation of an adaptive, specific T-cell response (95,102,107). Signals that provoke inflammation result in better adaptive immune responses, which is the basis of the "danger model" proposed by Matzinger (108). Inflammatory signals activate professional APC, such as dendritic cells, which can subsequently prime potent tumor-specific T-cell responses. Thus, several investigators have sought to enhance tumor-specific antigen presentation to T cells by using activated, *ex vivo*-generated DC (109-111). Pre-clinical animal studies and Phase I clinical trials have demonstrated that DC, pulsed with tumor lysates, RNA, and/or peptides, can elicit anti-tumor immune response against CNS neoplasms (74,75,112-114). Although the clinical data to date offers too limited information to make any conclusions about efficacy, the advantages of DC-based immunotherapy, along with its clinical safety and feasibility, make this a promising immunotherapeutic option. Despite such promise, however, there are still many practical and theoretical problems for the clinical development of DC-based vaccines. Currently, no consensus exists regarding the optimal techniques of DC generation, methods of DC loading (e.g., tumor lysates versus specific peptide epitopes), maturation status (e.g., immature versus mature), dose, or route of delivery. Also, the difficulties of immune response monitoring following dendritic cell vaccination are particularly acute in patients with brain tumors because, in contrast to patients with melanoma, there are few well-defined tumor antigens. Until advances in this domain occur and consensus is gained regarding methods of DC preparation and antigen loading, the rational potential of dendritic cell-based brain tumor immunotherapy has yet to be realized.

Investigators have also sought to activate host APC *in vivo* by delivering tumor-associated model antigens to the endogenous MHC class I pathway using recombinant viral or bacterial vectors, such as with recombinant *Vaccinia* or *Listeria* vaccinations (115-117). Direct *in vivo* infection of APC by viruses/bacteria engineered to express immunogenic tumor antigens could result in endogenous presentation of tumor epitopes in the context of MHC I, along with antigen processing via MHC class II pathways. This improved antigen processing/presentation, along with the danger signals associated with infection, may break tumor tolerance, enhance specific anti-tumor immunity, and induce epitope spreading (118,119). Immunotherapeutic strategies that elicit antigen cross-presentation and epitope spreading *in vivo* may be particularly beneficial for CNS tumors, because this phenomenon allows T cells to recognize shared endogenous tumor antigens along with the targeted epitope(s) (29,100,101,116). Given the heterogeneity of human brain tumors and the possibility of immune escape of tumor cells that do not express targeted epitopes, exploiting the process of determinant spreading will be useful in the clinical context of designing tumor vaccines in the future.

6. SUMMARY AND PERSPECTIVE

Much has been learned in recent years about the molecular and cellular interactions involved in anti-tumor immune responses. This is a lot of ground to cover, and this review article is by no means exhaustive of the field. We have, out of necessity, been somewhat selective about the information included. Nevertheless, we have touched upon a few key issues that will be fundamental to consider for future developments in cellular immunotherapy for brain tumors.

First, the CNS is not, like previously believed, refractory to the infiltration of immune cells and immune reactivity. The CNS may be an inefficient site to *initiate* immune responses because of active processes, anatomical constraints, and evolutionary adaptations designed to limit immune reactivity within a site where the benefits of immunity/inflammation are often outweighed by the harm caused (4). However, targeted immune responses can and do occur within the CNS to efficiently eliminate foreign antigens when properly activated. The ability of immune cells to track and target pathogenic or neoplastic antigens throughout the body makes the immune system inherently ideal for therapeutic manipulation in neuro-oncology. Harnessing the energy of the immune system to work to the benefit of patients with malignant brain tumors, however, will need to be carefully weighed against the problem of inflammation, which is an issue that distinguishes the CNS from other sites targeted for immunotherapy.

Another issue that distinguishes primary cerebral malignancies from other tumors, at least for the time being, is the paucity of defined tumor antigens that can be specifically recognized by T cells. Indeed, the difficulties of immune response monitoring are particularly acute in patients with brain tumors because, in contrast to patients with melanoma, there are few well-defined tumor antigens

for glioma. Therefore, unless advances in this domain occur, rational T cell-based brain tumor immunotherapy will continue to lag behind that for other cancers. With advances in "tumor immunomics," the identification of glioma-associated antigens should greatly help in the design of novel immunotherapeutic cancer vaccines for the future treatment of brain tumors.

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Abbreviations: CNS, central nervous system; GBM, glioblastoma multiforme; DC, dendritic cells; PAMP, pathogen-associated molecular patterns; MIC, MHC class I chain related; Rae-1, retinoic acid early inducible; ULBP, UL16 binding proteins; HSP, heat shock proteins; APC, antigen presenting cells; T_H, helper T lymphocyte; CTL, cytotoxic T lymphocyte; MHC, major histocompatability complex; TCR, T-cell receptor; LAK, lymphokine activated killer; TIL, tumor infiltrating lymphocyte.

Key Words: Neuro-Oncology, Brain Tumor, Glioma, T cell, Dendritic Cell, Immunomics, Immunotherapy, Review

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