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Consensus on the role of human cytomegalovirus in glioblastoma

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The human cytomegalovirus (HCMV) and glioma symposium was convened on April 17, 2011 in Washington, DC, and was attended by oncologists and virologists involved in studying the relationship between HCMV and gliomas. The purpose of the meeting was to reach a consensus on the role of HCMV in the pathology of gliomas and to clarify directions for future research. First, the group summarized data that describe how HCMV biology overlaps with the key pathways of cancer. Then, on the basis of published data and ongoing research, a consensus was reached that there is sufficient evidence to conclude that HCMV sequences and viral gene expression exist in most, if not all, malignant gliomas, that HCMV could modulate the malignant phenotype in glioblastomas by interacting with key signaling pathways; and that HCMV could serve as a novel target for a variety of therapeutic strategies. In summary, existing evidence supports an oncomodulatory role for HCMV in malignant gliomas, but future studies need to focus on determining the role of HCMV as a glioma-initiating event.

Keywords: cancer, DNA virus, gliomas, herpesvirus, human cytomegalovirus.

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†Human cytomegalovirus and gliomas symposium participants are listed in the Appendix.

This consensus statement is the culmination of a series of discussions held at an open meeting among researchers studying the impact of human cytomegalovirus (HCMV) in gliomas. Sponsored by Accelerate Brain Cancer Cure and the National Brain Tumor Society, this meeting convened in Washington, DC, in April 2011, and provided the opportunity for oncologists and virologists to freely discuss their most current data addressing this topic. Here, we report the consensus position in 4 major areas:

(1) existence of HCMV in gliomas,
(2) role of HCMV in gliomas,
(3) HCMV as a therapeutic target, and
(4) key future investigative directions.

Existence of HCMV in Gliomas

Detection of HCMV Proteins, Genes, and Nucleotides

The expression of HCMV proteins and oligonucleotides in a high percentage of gliomas was first reported by Cobbs et al. in 2002.[1] Since that time, controversy regarding the existence and role of HCMV in gliomas has been debated in the literature. An equal number of studies that specifically address the presence or absence of the virus in this disease have been published.[1–8] Documenting the presence of HCMV in gliomas has been confounded by the lack of a uniform operational definition of positivity in tumor tissues and the use of different methodological approaches. The approaches used

in these various studies were categorically addressed by Scheurer et al. in 2008. The authors described the necessity of optimizing sample preparation and detection techniques when extracting from paraffin-embedded tissues to adjust for low levels of infection. By doing so, they were able to detect the HCMV immediate early 1 (IE1) protein in 100% of glioblastomas and 82% of low-grade gliomas with use of immunohistochemistry.[7] They further reported detection of HCMV-specific oligonucleotides in the same areas of IE1 expression in the tumor, as determined by in situ hybridization. Their conclusion, as initially described by Cobbs et al. in 2002, is that HCMV IE1 and virus-specific oligonucleotides could be readily detected by optimizing these techniques. Moreover, they validated another of the findings by Cobbs and colleagues, which was the lack of detection of virus expression (or virus-specific oligonucleotides) in areas of necrosis or outside the tumor margin. These findings (Fig. 1) are consistent among studies that have described the ability to detect HCMV proteins and oligonucleotides.[1,3,7]

To date, immunohistochemistry, in situ hybridization, electron microscopy, polymerase chain reaction (PCR) coupled with DNA sequencing, enzyme-linked immunosorbent assay, and flow cytometry have been used to detect HCMV proteins and DNA in human glioblastoma tissue samples.[1,3,5,7–11] Collectively, these studies have identified the presence of the HCMV proteins IE1, US28, pp65, gB, HCMV IL-10, and pp28 and the HCMV genes *IE1* and *gB*. The most commonly studied protein has been IE1. Among studies with positive findings that used immunohistochemistry for the detection of IE1, one reported 16% of samples positive for this protein,[8] and the remainder were in the range of 93%–100%.[1,3,7,10] A control probe specific for detection of a herpes simplex virus oligonucleotide did not show positivity in glioblastoma samples.[1] No HCMV proteins or nucleotides were detected in normal brain controls, areas of normal brain adjacent to tumor, or in one HCMV-negative glioblastoma.[1,3,7,10]

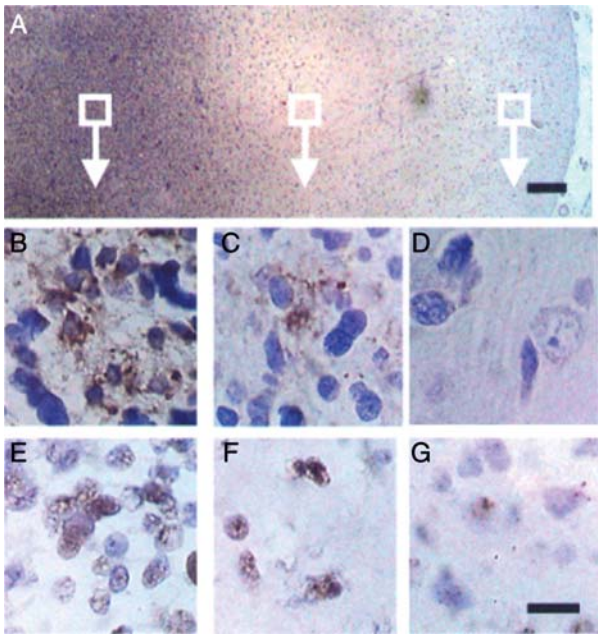

Fig. 1. Correlation of patterns of immunohistochemical localization of human cytomegalovirus (HCMV) immediate early 1 (IE1) protein with in situ hybridization for HCMV DNA in a glioblastoma (GBM) that invades normal brain. (A) Low-power view of anti-IE1 immunostain demonstrates GBM invading normal brain cortex (cortical surface at far right; bar, 200 μm). (B–D) Boxed areas in (A) at higher power demonstrate IE1 immunoreactivity moving from an area of frank tumor (B) to an area of invading tumor (C) to an area of normal brain (D). Detection of HCMV DNA by in situ hybridization using an HCMV total genome probe (in an adjacent section and similar regions of the same tumor in B–D) reveals a similar pattern, moving from malignant (E) to invasive (F) to normal (G) brain. Bar, 10 μm.

Sequencing the HCMV genome found in DNA isolated from human glioblastoma samples has proven to be challenging. Unpublished data presented by T. F. Kowalik demonstrated difficulty in sequencing complete genomic HCMV DNA from individual samples, possibly as a result of low copy numbers of viral DNA or of fragmented, discontinuous viral genomes. However, HCMV genomic DNA was detected in 94% of samples using a combined PCR-DNA sequencing methodology, a technique that revealed polymorphisms in certain regions of the glioblastoma-associated viral genomes, such as those encoding the tegument protein pp65. Because HCMV populations have been shown to be highly diverse in clinical specimens,[12] a possible explanation is that the HCMV genomes associated with glioblastoma are tumor-specific and derive from the diverse viral populations that exist in individuals. Cumulatively, on the basis of these findings and the results of studies describing activity of HCMV proteins in gliomas and glioma cell lines,[9–11,13,14] a consensus was reached that there is sufficient evidence to conclude that HCMV sequences and viral gene expression exist in most, if not all, malignant gliomas.

Lytic Versus Latent Disease

A second area of controversy is whether or not HCMV exists in a lytic or latent state in gliomas. Lytic HCMV infection is characterized by intranuclear expression of the IE genes *IE1* and *IE2*. Their gene products, along with those of the delayed early (DE) genes, regulate transcription of viral and host genes, which, in turn, drive viral replication. This process is facilitated by the inhibition of apoptosis mediated by these gene products.[15] When seen histologically, the presence of intranuclear viral protein inclusions, the classic "owl's eyes," confirms this diagnosis. This finding has not been observed in glioma specimens or in glioma cancer stem cells (gCSCs) (A.B. Heimberger, unpublished data). To date, no investigator has demonstrated the production of infectious HCMV virions by gliomas.

In contrast, latency is characterized by the carriage of the viral genome in the absence of both lytic gene

expression (which include the IE genes) and production of infectious virions.[16] The mechanisms that govern latency have not yet been explicitly defined, and only a small number of HCMV latency-associated transcripts have been identified.[16] Expression of HCMV latency-associated transcripts has not yet been measured in gliomas, but the ubiquitous expression of *IE1* implies that HCMV does not reside in these tumors in a truly latent state.

The existence of HCMV in gliomas does not appear to fit classic definitions of lytic or latent disease. Typically, HCMV lytic infection results in productive replication, significant cellular damage, and frequently, cell lysis—referred to as the cytopathic effect. However, known tumor viruses are typically latent, and some replicate in the host cell using host cellular proteins without producing infectious virions.[17] Given these facts, a model similar to that proposed for the role of HCMV in the development of cardiovascular disease could be considered for gliomas. Here, it was postulated that persistent infection of endothelial cells by HCMV plays a role in the development of hypertension.[18] Persistent infection, as demonstrated by viral gene expression without cytopathic effect, led to production of inflammatory cytokines and renin, which resulted in the development of hypertension in an in vivo model. Applying this model to gliomas, persistent infection could result in the expression of HMCV genes, leading to production of cytokines that contribute to pathogenesis or of proteins known to disrupt cell-cycle regulation. Such candidate genes and products are outlined in more detail in later sections of this article. Furthermore, HCMV can encode $\geq$166 genes, not all of which have been extensively studied.[15] In the context of tumor viruses, those expressed during latency could be the most significant, relative to gliomagenesis.

Epidemiology

The seroprevalence of HCMV in the general population is up to 80%,[19] in contrast with the prevalence of glioblastoma of 0.0257%.[20] To date, no epidemiological correlation between the timing of HCMV infection and the subsequent risk for development of gliomas has been reported. In reviewing the epidemiology of tumor viruses, many factors other than simple association influence the development of cancers associated with these viruses. As examples, the seroprevalence of human papillomavirus and Epstein Barr virus exceeds the incidence of cervical cancer or Burkitt's lymphoma, and the development of liver cancer is associated with a combination of carcinogenic exposure and either hepatitis B virus or hepatitis C virus infection.[17] Therefore, a proposed role for HCMV in gliomagenesis is most likely to be associated with a yet undefined event.

Two unique hypotheses are postulated: one presented at the meeting and one recently published. On the basis of studies showing that HCMV enters the cell via PDGFRα[21] and that specific PDGFR haplotypes are associated with a greater incidence of glioblastoma,[22] A.B. Heimberger suggested that there may be PDGFR receptor haplotype differences that confer differential susceptibility to HCMV infection of glioma cells where the receptor is overexpressed or present on a glioma cell of origin. Alternatively, a recently published proposal suggested that host genes that would affect binding affinity to an HCMV-encoded Fc γ receptor (FcγR) could serve as a risk factor for gliomagenesis. HCMV FcγR is involved in the ability to evade immune detection by interfering with antibody-mediated cellular toxicity. Thus, individuals possessing FcγR receptors with different binding affinities may have variable capability for clearing virally infected cells.[23]

Role of HCMV in Gliomas

Oncomodulation

The most accepted concept discussed at the meeting is that there is sufficient evidence to support the hypothesis that HCMV could modulate the malignant phenotype in glioblastomas.

The concept of HCMV and oncomodulation was first proposed by Cinatl et al. in 1996, who provided evidence that, although HCMV could modulate the malignant properties of cells, it was not directly involved in transformation.[24] Earlier studies have described the transforming capability of HCMV in rodent and human cells, but its DNA was not retained in these cells, and the presence of HCMV antigens, although initially demonstrated, decreased with subsequent passage.[25] At present, HCMV is not considered to be an oncogenic virus. Features attributed to known oncogenic viruses (Table 1) have not been identified in HCMV-infected gliomas, such as sustained expression of oncoproteins or genomic integration. Genetic mapping of gliomas as a means of establishing tumor phenotype has not shown HCMV gene products, but these platforms did not include HCMV genes.

In 2000, Hanahan and Weinberg described 6 essential alterations in cell physiology that are the hallmarks of cancer, including (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) activating invasion and metastasis, (4) enabling replicative immortality, (5) inducing angiogenesis, and (6) resisting cell death.[26] These criteria were recently updated in March 2011 to include (7) deregulating cellular energetics, (8) avoiding

Table 1. Basic mechanisms of established tumor viruses

Virus	Oncogenic Mechanism
Human Papilloma Virus	Oncoprotein, Integration into host genome
Hepatitis B Virus	Oncoprotein, Integration into host genome
Epstein Barr Virus	Oncoprotein, Translocation signature
Human Herpesvirus-8 (Kaposi's Sarcoma)	Oncoprotein

Table 2. Overlap of Human Cytomegalovirus (HCMV) biology with altered cellular physiologies classified as hallmarks of cancer

Cancer Hallmark	HCMV Activity	HCMV Protein Involved
Sustaining Proliferative Signaling	Induces cell cycle progression to S phase Induces expression of E2F genes Phosphorylates Rb	IE2 pp71 UL97
Evading Growth Suppressors	Activates EGFR Dysregulates Cyclin E expression Inhibits p53 degradation Decreases levels of p21 Induces expression of p53 Binds to p53	HCMV infection IE1 mtrII
Activating Invasion and Metastasis	Activation of RhoA dependent motility of U373 cells Activates smooth muscle cells	US28
Enabling Replicative Immortality	Activation of telomerase	IE1
Inducing Angiogenesis	Induction of VEGF expression Induction of IL-8	US28 IE1
Resisting Cell Death	Inhibits apoptosis Activates PI3-K/Akt pathway	IE1 IE2 vMIA, vICA
Deregulating Cellular Energetics	Increases flux through glycolytic pathway, acetyl CoA, flux of carbon, nucleotide biosynthesis	HCMV infection
Avoiding Immune Destruction	Production of homologs to immunosuppressive cytokines Inhibits expression of MCH I Intracellular retention of NKG2D Induces expression of TGF-β1	HCMV IL-10 US2 UL16 IE2
Genome Instability and Mutation	Chromosome damage Inhibits DNA damage repair Increases mutation frequency Induces chromosome aberrations in cell lines	Unidentified protein HCMV infection pp65 and pp71 IE1 UL76
Tumor Promoting Inflammation	Induces production of RANTES, fraktalkine, MCP-1 NF-κB activation & IL-6 production	HCMV infection US28

immune detection, (9) genome instability, and (10) mutation- and/or tumor-promoting inflammation.[27] Cobbs, Alwine, and Kalejta presented studies showing an overlap of HCMV biology with the essential alterations of cell physiology that are hallmarks of cancer (Table 2).[10,11,28–60] On the basis of these publications and the findings that we outline below, we consider these altered physiologies with respect to glioblastoma and how HCMV biology can enable the characteristics of cancer and function as an oncomodulator.

Sustaining Proliferative Signaling, Evading Growth Suppressors, and Enabling Replicative Immortality

The PDGFRα polypeptide is a strong candidate as the portal of access of HCMV into malignant glioma cells or their cells of origin. The HCMV envelope protein gB has been shown to directly interact with and to phosphorylate this receptor.[21] Furthermore, viral entry into the cell was shown to activate the PI(3)K pathway with induction of Akt, with none of these events being detected after either blockade of the receptor or deletion of the gene encoding it. Although focal gene amplification and expression of PDGFRα is highest in the proneural phenotype, amplification of PDGFRα is seen in all phenotypes of glioblastoma.[61]

To evaluate the effects of persistent IE1 expression in glioblastomas, stable expression of this viral protein in human glioblastoma cell lines (U87, U251, LN229, UL118) was evaluated.[14] Such expression demonstrated differential effects on cellular proliferation, such that in some cases, it was increased and, in others, unaffected or decreased. MAPK and AKT signaling was considered as a possible mechanism to explain this finding and was found to show a sustained increase. Another important finding was the increase in phosphorylation of the cell-cycle regulator Rb in all the malignant glioma cell lines studied. The inactivation of Rb by HCMV in nonglioblastoma cell lines has been described elsewhere in the virology literature, whereby 2 different viral proteins both degrade and phosphorylate the tumor suppressor.[36,38] Numerous studies have documented HCMV interference with the cell cycle, showing p53 and Rb being targeted, as reviewed in Michaelis et al.[43] Although not extensively studied in glioblastomas, this represents an important area for further study.

In 2009, Straat et al. reported constitutive telomerase reverse transcriptase (hTERT) expression and telomerase activation as a result of HCMV infection in multiple malignant glioma cell lines.[11] To examine a potential mechanism for the activation of telomerase subsequent to HCMV infection, the authors evaluated the behavior of one of its regulators, the transcription factor specificity protein 1 (Sp1). ChIP analysis in fibroblast cells (MRC 5) indicated that Sp1 and IE1 were bound to the hTERT promoter after HCMV infection. When human glioblastoma tissue samples were examined

using immunohistochemistry, a direct correlation between immediate early antigen (IEA) and hTERT expression was seen in all 10 patient samples.

Activating Invasion and Metastasis

HCMV infection of U87 cells was found to enhance focal adhesion kinase activity, in addition to significantly increasing cell migration relative to that in immortalized human astrocytes.[13] This suggests that HCMV can be associated with a more aggressive phenotype of glioblastoma.

Inducing Angiogenesis

US28 expression has been identified in glioblastomas, primarily in vascular endothelial cells.[10] It is a constitutively active HCMV-encoded G protein-coupled receptor expressed with early-stage kinetics during infection. It has gene sequence homology to human chemokine receptors CCR1and CX3R and is capable of binding chemokines CCL2, CCL5, and CX3CL1, among others.[62] Stable expression of US28 in NIH 3T3 cells resulted in a significant increase in IL-6 and VEGF production,[10] and implantation of these cells in nude mice resulted in tumor formation.[63] The intracellular effects of US28 are multiple and include upregulation of cyclin D1[63] and NF-κB.[10] The NF-κB activity increased production of IL-6, which activated the signal transducer and activator of transcription 3 (STAT3) in both an autocrine and paracrine manner.[10] STAT3 has previously been described as a key molecular hub of tumorigenesis[64,65] and immune suppression, especially in gliomas.[66] Its induction in the neural progenitor cells of mice has been shown to induce high-grade gliomas, along with increased VEGF expression and angiogenesis.[67]

Genomic Instability and Interference with DNA Damage Response

Human neural progenitor cells are fully permissive to HCMV infection, which results in premature and abnormal differentiation.[68] As a consequence of infection, altered attachment, migration, loss of multipotency, and down-regulation of MIR21, OLIG 1, and SOX2 have been described.[68] In the context of persistent infection of vulnerable stem cells, HCMV-mediated genomic injury could promote oncogenesis, because HCMV has been shown to induce specific chromosome damage.[69] Purified virions from 3 different strains of HCMV were found to reproducibly induce breaks in chromosome 1 at 1q42 and 1q21 in 2 cell types (foreskin fibroblasts and human embryonic lung cells). Although this event does not occur at high frequency, it does occur reliably (E. A. Fortunato, personal communication). The loss of at least 1 copy of the chromosome 1q42 band has been reported in a small percentage of patients with glioma.[70]

To date, HCMV interference with the DNA damage response has not been studied in malignant gliomas, although such an interaction has been documented in the virology literature.[33,35,71,72] Although this interaction is to facilitate viral replication, an occurrence in parallel with an appropriate genetic mutation could favor development of a glioblastoma.

Tumor-Promoting Inflammation and Avoidance of Immune Detection

A feature common to both HCMV virulence and glioblastoma malignancy is the ability to evade immune detection. HCMV uses several mechanisms to evade the host-cell immune response and promote immune suppression (Fig. 2).[73–77] HCMV has also been shown to promote a chronic inflammatory state associated with increased expression of ROS, RNS, and COX-2.[78–82]

In most healthy individuals, HCMV remains latent throughout the lifetime of the host. Bone marrow CD34+ progenitor cells have been identified as one site of HCMV latency, and the latent viral genome is carried through the myeloid lineage as these cells differentiate.[83] Terminal differentiation of immature myeloid cells into mature macrophages or dendritic cells in the

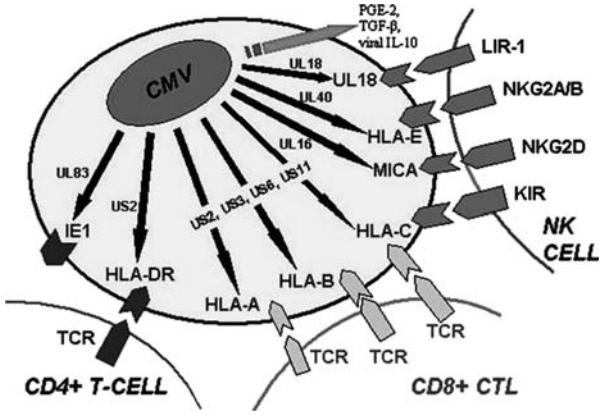

Fig. 2. Selected immune subversive human cytomegalovirus (HCMV) proteins blocking CTL and NK cell recognition and antigen presentation pathways. HCMV gene nomenclature designates genes as UL for unique long and US for unique short to reflect regions of the genome from which the gene originates. UL83 (pp65) inhibits presentation of the immunodominant CMV protein immediate early 1 (IE1); US2 mediates degradation of HLA class I and II a chains; US3 causes retention of class I molecules within the ER; US6 inhibits TAP-mediated peptide transportation into the ER; US11 (like US2) causes destruction of class I a chains; UL16 inhibits NK cell recognition via the activating receptor NKG2D by binding to its ligands (ULBPs); UL18 activates LIR-1, an inhibitory receptor found on NK cells, lymphocytes, and most other immune cells; and UL40 activates the inhibitory NKG2A/B receptor by upregulating HLA-E expression. HCMV produces a functional IL-10 homolog (UL111A) and induces expression of cellular PGE-2 and TGF-β, which further inhibit NK cell response.

context of inflammation and immunosuppression has been shown to reactivate the virus.[16,84–86] Recent evidence suggests that glioblastoma tumor-associated macrophages and microglia are infected with HCMV.[9] This population of cells is a major component of the tumor microenvironment.

HCMV can induce a unique M1/M2 polarization signature that promotes viral dissemination and persistence, a process involving the induction of IL-6 and TNF-α.[87,88] These cytokines would be expected to contribute to an oncogenic microenvironment, because chronic expression of TNF-α and IL-6 is directly linked to oncogenic transformation in inflammation-induced animal models of cancer.[89] Autocrine mechanisms may also exist, considering that TNF-α has been implicated in the reactivation of HCMV in immunosuppressed transplant recipients through enhancement of HCMV IE promoter activity.[90] Whereas these cytokines are prototypical of the M1 type proinflammatory cascade, HCMV also simultaneously induces the immunosuppressive M2 type macrophage responses. Recently, it was reported that glioma cancer stem cells (gCSCs) harvested from human glioblastomas produce HCMV IL-10.[9] This viral homolog of the human IL-10 immunosuppressive cytokine induces the M2 phenotype that has been described in glioblastoma-associated macrophages and microglia.[91] As such, a feed-forward mechanism is proposed in which the HCMV-induced M2 macrophages/microglia produce increased VEGF and TGF-β and stimulate gCSC migration. This study also identified specific glioblastoma cellular subpopulations harboring HCMV, the gCSCs, and cells of monocyte lineage. In monocytes harboring HCMV, IE1 expression was induced after exposure to HCMV IL-10, which further potentiates the feed-forward mechanism.

HCMV as a Therapeutic Target

In Vivo Mouse Model

A recently developed preclinical model of murine CMV (MCMV) was presented by Kwon and Chiocca that could be used to test potential anti-HCMV therapeutics. In this model, transgenic (Mut3) mice were engineered to develop spontaneous gliomas that were then perinatally infected with MCMV. Mice that developed gliomas in the MCMV cohort exhibited more aggressive tumors and showed a marked decrease in median survival time, compared with the uninfected control cohort. This suggests that MCMV infection may accelerate glioma progression.

Clinical Trials: Valganciclovir and Tumor Vaccination

Results are pending from a phase I/II double-blind randomized clinical trial of valganciclovir administered to patients with gliomas as a postsurgical add-on therapy performed at the Karolinska Institute. Valganciclovir is a nucleoside analog and targets HCMV replication through the disruption of DNA synthesis. If a survival advantage is identified, it will be valuable to know whether it is the result of reduced activity of HCMV or of the activity of the drug in disrupting DNA synthesis in actively dividing tumor cells or a combined effect. Because the pathology of HCMV in gliomas does not recapitulate what is seen in active, lytic infections, it is not clear what the trial will demonstrate. However, the reporting of pathological and clinical correlates subsequent to valganciclovir treatment will provide important clues to viral contribution to disease, mechanisms of therapy, and potential interactions between antivirals and other chemotherapeutics.

A second therapeutic strategy targeting HCMV antigens expressed in glioblastomas was presented by Dr. Mitchell from Duke University. A phase I/II autologous dendritic cell vaccine pulsed with HCMV peptides in patients with newly diagnosed glioblastoma multiforme showed a median survival time of 21 months. A follow-up phase II clinical trial of patients with newly diagnosed glioblastoma multiforme is targeted to start by the end of 2011 and will consist of vaccinating patients with multiple HCMV peptides sequentially, along with temozolomide treatment in a manner similar to that used in the epidermal growth factor variant III peptide clinical trials.[92,93] The feasibility of this type of approach is supported by a phase I clinical trial that investigated the use of vaccination with autologous dendritic cells pulsed with autologous tumor lysate. In this trial, a patient developed a robust HCMV-specific $CD8^+$ T-cell response to the pp65 HCMV immunodominant epitope that began immediately after one injection of autologous tumor lysate-pulsed dendritic cells.[5] Because HCMV proteins have not been found to be expressed outside the confines of tumor tissue, responses are not expected to target uninvolved surrounding brain tissue.

Although a specific role for HCMV in gliomas remains to be defined, there was agreement that it could serve as a novel target for a variety of therapeutic strategies.

Key Future Investigative Directions

Epidemiology and Risk Factors

No epidemiological study to date has been undertaken to ascertain why such a small percentage of the population with latent HCMV develops gliomas. It is unknown whether there are additional risk factors that predispose patients with glioma to the development of their disease, such as genetic polymorphisms that render susceptibility to the oncomodulatory effects of HCMV. Studies addressing possible genetic factors, such as the PDGFR haplotype, FcγR, or environmental factors need to be conducted to identify risk factors and to further elucidate the mechanisms involved in the role of HCMV in glioma pathology. Efforts are under way to develop a HCMV vaccine to prevent congenital birth defects, and ultimately, this cohort could be followed up longitudinally to ascertain the risk of glioma development.

Identification of Therapeutic Targets

Further elaboration of how HCMV contributes to glioma malignancy could identify novel therapeutic targets. Although a portal for cellular entry has been identified,[21] how HCMV gains access to the central nervous system is unknown. It is possible that the virus is trafficked and introduced to the tumor milieu via circulating monocytes and that the immunosuppressive glioma microenvironment stimulates reactivation of disease, perpetuating a feed-forward mechanism, but this does not address the initiating event. A blockade of this mechanism could potentially reduce the aggressive nature of glioblastomas. Furthermore, there may be only specific HCMV strains that can initiate gliomagenesis. Irradiation of live HCMV renders the virus noninfectious. Does the same occur in the context of glioma? Does HCMV infect neural, glial, or glioma progenitor cells at an early stage of gliomagenesis? Would this allow an opportunity for immune clearance or immediately trigger immune suppression during early stages of gliomagenesis?

As data from the aforementioned clinical trials become available, it could serve as a platform in the formulation of hypotheses to address these questions.

Conclusions

Sufficient evidence has emerged to suggest that HCMV could modulate the malignant phenotype in glioblastomas, and elements of its biology overlap those considered to be hallmarks of cancer. Recent evidence supports the continued development of therapeutic HCMV vaccine to reduce glioblastoma's malignancy. Studies of the mechanisms used by HCMV should include a major initiative to understand the contributions of HCMV to gliomagenesis.

Conflict of interest statement. None declared.

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Appendix

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