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Review

Human cytomegalovirus-mediated immunomodulation: Effects on glioblastoma progression

Haidn Foster[a], Ilya V. Ulasov[a,b,*], Charles S. Cobbs[a,**]

[a] Swedish Neuroscience Institute, Center for Advanced Brain Tumor Treatment, Seattle, WA 98122, USA
[b] Institute of Molecular Medicine, I.M. Sechenov 1st Moscow State Medical University, Troubetskaja str. 8, Building 2, Moscow, 119991, Russia

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ABSTRACT

The presence of human cytomegalovirus (HCMV) and glioblastoma multiforme (GBM), first established in 2002, has developed into an area of considerable interest and controversy. Numerous studies have found evidence of possible HCMV infection of GBM tumor cells as well as myriad onco- and immunomodulatory properties exhibited by HCMV antigens and transcripts, while recent reports have failed to detect HCMV particles in GBM and question the virus' role in tumor progression. This review highlights the known immunomodulatory properties of HCMV, independent of GBM infection status, that help drive the virus from peripheral blood into the vital tissues and subsequently dampen local immune response, assisting GBM tumors in evading immune surveillance and contributing to the disease's poor prognosis. Emerging antiviral approaches to treating GBM, including antiviral drugs and immunotherapies directed against HCMV, are also examined.

1. Introduction

Glioblastoma multiforme (GBM), a World Health Organization grade IV malignant astrocytoma, is the most common and aggressive primary brain cancer, with a median survival time of approximately 14 months following diagnosis and a < 3% survival rate at 5 years [1,2]. GBM may develop from lower-grade gliomas (10% of cases) or arise *de novo* (90%) but in either case is virtually incurable [3]. The standard of care for newly-diagnosed GBM includes surgical resection followed by radiotherapy in addition to concomitant and adjuvant temozolomide, while antivirals and immunotherapy have also emerged as promising adjunct therapies in improving clinical prognosis for GBM patients [4].

Human cytomegalovirus (HCMV) is a β-herpesvirus that infects a majority of adults in the United States and can result in serious disease in infants and the immunocompromised such as AIDS and transplant patients [5,6]. In immunocompetent hosts, however, initial infection tends to present subclinically, followed by lifelong latency with the potential for reactivation. HCMV can infect numerous cell types but is most often found in cells of myeloid lineage such as monocytes and their progeny: dendritic cells (DC) and macrophages [6,7]. While the virus remains latent in monocytes, productive lytic infection is observed following cell differentiation. Consistent with other herpesviruses, HCMV gene expression consists of a temporal cascade of immediate-early, early, and late stage transcription throughout the course of productive infection. Immediate-early proteins mediate cellular and viral gene expression; early proteins are critical for viral DNA replication; and late proteins are mostly structural and used to assemble the viral capsid.

2. HCMV detection in glioblastoma

While it is not known to be an oncogenic virus, cytomegalovirus has been detected in colorectal, prostate, and skin cancers, as well as myriad intracranial tumors [8–11]. HCMV was first discovered in the tumors of immunocompetent GBM patients in 2002 [12]. The viral proteins immediate-early 1 (IE1) and phosphoprotein 65 (pp65) have since been identified in 50–100% of glioblastoma specimens, and HCMV nucleic acids have been found in $IE1^+$ and $pp65^+$ GBM cells [12–17]. In these studies, tumor-adjacent normal brain consistently displayed no evidence of infection. Still, several recent reports have disputed HCMV's presence in glioblastoma tumor cells and posit that the virus may not significantly correlate with GBM [18–20]. While the difficulty of detecting HCMV in GBM cannot be discounted, none of these studies employed the preparatory protocols previously published by our group that we have found necessary for sensitive detection of

* Correspondence to: I.V. Ulasov, Ben & Catherine Ivy Center for Advanced Brain Tumor Treatment, Swedish Neuroscience Institute, 550 17th Avenue, Seattle, WA 98122, United States.
** Correspondence to: C.S. Cobbs, Swedish Neuroscience Institute, 550 17th Avenue, Seattle, WA 98122, United States.
E-mail addresses: Ilya.Ulasov@Swedish.org (I.V. Ulasov), Charles.Cobbs@Swedish.org (C.S. Cobbs).

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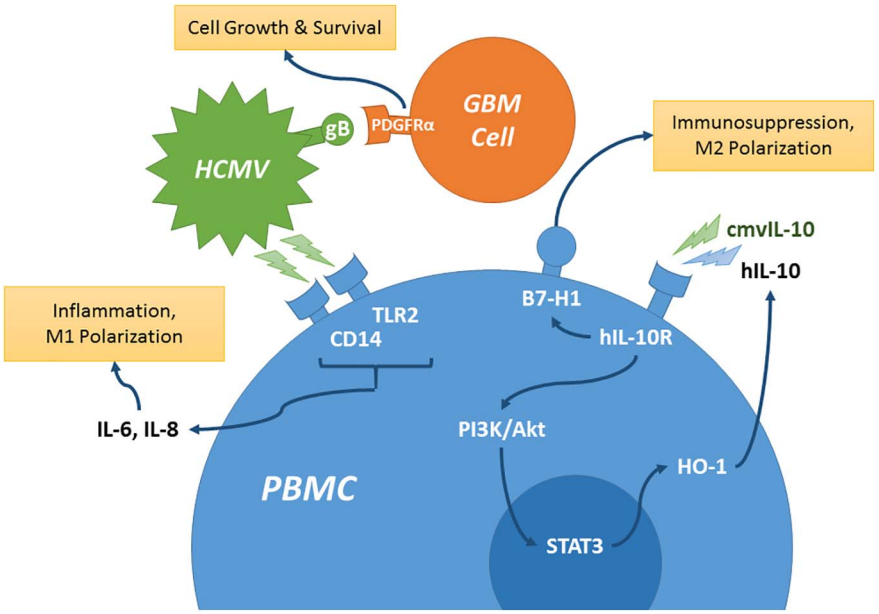

Fig. 1. Interaction of HCMV virion, GBM cell, and a representative leukocyte. HCMV presence stimulates CD-14 and TLR-2 receptors, inducing production of inflammatory cytokines IL-6 and IL-8. Binding of HCMV surface protein gB to the receptor PDGFRα initiates a signaling cascade within GBM cells, resulting in increased cell growth and survival. The anti-inflammatory cytokine cmvIL-10 and its human homolog hIL-10 induce expression of B7-H1 (a.k.a. PD-L1), leading to immune suppression.

low copy viral particles in GBM [21,22].

Though HCMV detection in GBM cells continues to be contested, positive association between levels of viral particles detected in glioma tissue samples and tumor aggressiveness, as well as poor clinical prognosis, has been demonstrated by multiple groups. For instance, GBM specimens were found to contain 79% HCMV antigen-positive cells by IHC while only 48% of cells from lower-grade gliomas stained positive [15]. GBM patients with < 25% HCMV-positive tumor cells also lived an average of 20 months longer and exhibited delayed tumor progression compared to those with higher levels of infected cells [16]. Remarkably, a GBM patient enrolled in a vaccine trial using DCs pulsed with autologous tumor lysate developed a vigorous response to HCMV pp65 after a single infusion, resulting in the patient's total complement of pp65-specific CD-8$^+$ T cells rising from 0.2% to 4.4% [23]. Considered together, these correlations strongly suggest against false positive detection of virus in GBM tissue due to issues of sample contamination, incident infection, or unsuitable probe concentration.

3. Effects of proximal HCMV virions on glioblastoma

Though there are several oncomodulatory effects known to result from HCMV infection of GBM tumor cells *in vitro*, including increased stemness potentiated by HCMV micro RNA, the virus can influence cell behavior—even contributing to neoplastic immortalization and development of a malignant phenotype—without directly infecting cells [24]. CD14 and toll-like receptor 2 (TLR2), for example, induce production of proinflammatory cytokines such as IL-6 and IL-8 in the presence of extracellular HCMV virions [25]. Further evidence for the extracellular influence of HCMV is seen when the viral envelope glycoprotein B (gB) binds platelet-derived growth factor receptor alpha (PDGFRα), a tyrosine kinase receptor and the product of the third-most amplified gene in studied GBM samples [26]. PDGFRα, which is known to play a role in HCMV gene expression and may be required for the virus' entry into the cell, is phosphorylated upon binding gB, activating the phosphatidylinositol 3-kinase (PI3K) signaling pathway—another critical mediator of viral gene expression—which in turn activates Akt, inhibiting apoptosis [27–29]. The activated PI3K/Akt pathway promotes survival in several types of cancer, including multiple glioblastoma cell lines [30]. While PDFGRα activation is not necessarily required for GBM development, its persistent activation in neural stem cells results in tumor-like growths with characteristics of early-stage glioma [31].

4. Inflammation and immunosuppression: HCMV's dual action following infection

HCMV must ultimately infect cells to replicate irrespective of its ability to influence gene expression and behavior extracellularly. To that end, the virus induces the release of proinflammatory cytokines during its prereplicative phase and at strategic points during replication [25]. These cytokines recruit monocytes and neutrophils to the site of infection, after which monocytes harbor latent virus and neutrophils disseminate infectious virus throughout the host. As monocytes differentiate into macrophages, they preferentially polarize along a spectrum of phenotypes ranging from inflammatory and immune-activating (M1) to anti-inflammatory and immunosuppressive (M2). Once circulating monocytes are infected with HCMV, they release inflammatory cytokines such as TNF-α, IL-1, and IL-6 which induce polarization to an M1 phenotype [32]. M1 macrophages can drive infected cells from peripheral blood into the tissues, causing further inflammation in organ systems such as the colon and lungs in immunosuppressed individuals, thereby increasing infectious vectors throughout the host. The type of chronic, “smoldering” inflammation mediated by HCMV infection helps convert early neoplasia to an invasive, aggressive tumor phenotype resistant to both adaptive immunity and chemotherapeutic agents [33–35].

Once the virus has spread throughout the body, HCMV will inhibit the local immune response to escape surveillance, and in so doing will contribute to the immune evasion of infected and nearby cells (Fig. 1). It is known that *in vitro* infection of glioma cancer stem cells (gCSC) by HCMV induces the release of interleukin cmvIL-10, a homolog and inductor of human IL-10 (hIL-10) that is associated with higher-grade gliomas and may exacerbate the tumor's invasive potential, presenting a potential therapeutic target for GBM [36–38]. cmvIL-10 may additionally contribute to oncogenesis and immunosuppression by activating PI3K and phosphorylating the transcription factor STAT3 [39,40]. Activation of these pathways is necessary for cmvIL-10 to upregulate hIL-10, and can both induce TAMs to adopt an M2 phenotype as well as inhibit DC efficacy [37,41]. cmvIL-10 also downregulates proinflammatory cytokine production, inhibits maturation of DCs and promotes expression of immune inhibitors TGF-β and B7-H1, a ligand of PD-1 that stimulates CD8 + T cell death and inhibits apoptosis of regulatory T cells (Tregs) [36,37,42]. This potent immunosuppressive activity mimics the natural behavior of GBM-mediated immunoresistance in which glioma cells upregulate hIL-10, which