Several investigators have now demonstrated the expression of genes unique to Cytomegalovirus (CMV) in malignant gliomas. Many of these genes promote oncogenesis, alter tumor microenvironment, and serve as immunologic targets. Is the level of CMV infection within tumor cells sufficient to drive important oncogenic or immunosuppressive processes? Can CMV serve as a target for therapeutic intervention?
In this issue of *Clinical Cancer Research*, Dziurzynski and colleagues (1) report that CD133⁺ glioma stem cells (gCSCs) express human CMV proteins and secrete CMV interleukin (IL)-10, a viral cytokine homologue of IL-10 that is capable of converting the widespread monocytes and microglia in glioblastomas (GBMs) to an immunosuppressive tumor-supportive phenotype.

CMV is an endemic β-∗Herpesvirus* that does not usually cause significant clinical disease. However, CMV can cause fetal encephalitis and significant problems in immunocompromised adults. *Herpesviruses* have also been implicated in a number of human malignancies including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi’s sarcoma. Recently, expression of proteins unique to CMV has been reported within a large proportion of malignant tumors including colorectal carcinoma, prostate cancer, and gliomas (2, 3).

CMV induces numerous molecular changes within infected cells that could contribute to an oncogenic phenotype (3). For example, CMV has been shown to increase production of angiogenic factors, create a chronic inflammatory environment, and mediate numerous immunosuppressive changes in host cells. Furthermore, CMV infected cells exhibit increased cellular motility and invasion, reduced p53 and Rb function, elevated levels of telomerase, and increased resistance to chemotherapy-induced apoptosis. While viral infection has not been demonstrated to directly result in tumor formation, the CMV genome encodes several proteins that have been demonstrated to possess direct transforming capability when stably expressed in normal cells.
Cobbs et al. (2) first identified CMV antigen expression in GBM. These authors demonstrated through a series of optimized protocols the expression of CMV proteins (IE1, pp65, late antigens) by IHC in 27 out of 27 specimens but not within surrounding normal brain or other brain pathology specimens. Detection of CMV nucleic acids within these tumors was demonstrated using in situ hybridization and nested PCR confirmed by DNA sequencing. Our group (4) elaborated upon this work and confirmed expression of IE1 and pp65 in greater than 90% of GBM specimens by IHC, detection of early and late viral proteins (IE1, pp65, gB) in GBM primary cultures, and detection of nucleic acids using PCR (gB, pp65, and IE1) coupled with confirmatory DNA sequencing. Independent analyses by other groups (5, 6) have also confirmed CMV antigen expression in these tumors, including the expression of US28, a viral gene recently implicated in tumorigenesis in a transgenic mouse model of colorectal cancer (7, 8).

Dziurzynski et al. (1) investigated whether CMV infection could contribute to the immunosuppressive microenvironment characteristic of GBM. Tumor associated macrophages (TAMs) exposed to factors such as IL-10 can acquire M2 properties that suppress immune responses and support proliferative ones, thereby enhancing malignant growth. Herein Dziurzynski and colleagues demonstrate that TAMs derived from single cell GBM digests express CMV antigens. Additionally, examination of established CD133\(^+\) gCSCs revealed that CD133\(^{\text{high}}\) gCSCs preferentially express CMV antigens and secrete viral IL-10. Thus, neural precursor cells, cells with self-renewal capacity, and monocytes are permissive to CMV infection and could function as reservoirs for CMV reactivation within these tumors. In vitro exposure of CD14\(^{+}\)
monocytes to viral IL-10 downregulates expression of major histocompatibility complex (MHC) II and costimulatory molecule CD86 and upregulates expression of the immune inhibitory molecule B7-H1 as well as increases intracellular TGF-β, vascular endothelial growth factor (VEGF) and phosphorylated signal transducer and activator of transcription p-(STAT)-3. In return, viral IL-10-exposed monocytes increase gCSC migration. Therefore, in vivo exposure to viral IL-10 secreted by gCSCs could induce gCSC migration and an immunosuppressive milieu (Figure 1).

While the presence of CMV in gliomas has been reported by several independent laboratories, other groups have reported a failure to detect the presence of CMV within these tumors (9, 10). This discrepancy is likely attributable to methodological differences, as we and others have demonstrated that sensitive and optimized protocols are required to detect the very low levels of CMV likely present within these tumors. This observation begs the following questions: (1) is the level of CMV infection within tumor cells sufficient to drive important oncogenic or immunosuppressive processes and (2) is CMV is a clinically relevant target within these tumors for therapeutic intervention?

Koch’s postulates are a stringent set of criteria used for determining whether an infectious agent causes a disease. However, these strict criteria are not often met in cases of virally-induced cancers(11). The long latency between infection and cancer diagnosis and the fact that only a small minority of individuals exposed to a cancer-promoting virus (i.e. human papillomavirus (HPV) or Hepatitis B) ever develop a malignancy associated with the viral infection, clearly implicate multiple factors in addition to the infectious insult in the oncogenic process. In the majority of cancers
caused by viral infections the viral DNA is present in very small copy number, usually less than one DNA copy per 10 tumor cells. Furthermore, it is now clear that chronic infections can facilitate oncogenesis through indirect mechanisms without coding for transforming genes themselves, such as *H. pylori* promotion of gastric cancer and gastric lymphoma, providing several mechanisms by which infections may promote cancer formation. Finally, microbial genomes within cancer cells are often altered such that viable infectious pathogens are not recoverable from tumor cells, failing to fulfill major criteria for Koch’s postulates. Future experiments that demonstrate true modulation of the oncogenic phenotype via physiologic levels of infection with CMV-associated tumors will be pivotal in assessing any direct or indirect causality between CMV and oncogenesis.

Regarding immunological impact, as reported by Dziurzynski *et al*., would the levels of CMV viral IL-10 produced by *in vivo* gCSCs be sufficient to drive the immunosuppressive changes described within TAMs? If so, treatments targeting viral IL-10 could constitute novel and highly relevant therapeutic modalities. Similarly, evidence already exists that low levels of CMV viral gene expression may be sufficient for immunologic targeting (5), and it has also been demonstrated that killing by cytotoxic T lymphocytes can occur with presentation of as few as three antigenic peptides upon the surface of the target cell (12). Furthermore, if gCSCs do preferentially express CMV antigens *in vivo*, CMV-targeted therapies may profoundly inhibit malignant growth through eradication of this tumor propagating population. The work of Dziurzynski *et al* exemplifies that the continued characterization of biologically relevant levels of CMV infection as related to malignant progression and examination of CMV antigens as
immune-mediated targets represent important and potentially promising areas of future research.

**Figure Legend**

**Figure 1. CMV in GBM Tumor Microenvironment.** *Monocytes:* Peripheral blood monocytes harboring latent or reactivated virus infiltrate malignant gliomas where they differentiate into tissue associated macrophages (TAMs). Alternatively, gliomas already harboring reactivated CMV infection may infect monocytes upon entry into the tumor microenvironment.

*Glioma Stem Cells (GSCs):* GSCs enriched for CMV infection produce secreted vIL-10 which converts TAMs into an immunosuppressive and tumor propagating phenotype.

**CMV in tumor microenvironment:** Subclinical CMV infection and expression of viral gene products may contribute to enhancing several of the tumorigenic properties of malignant glioma including cellular migration and invasion, cellular proliferation, and resistance to cytotoxic therapy. Furthermore, the characteristic immunosuppressive phenotype in patients with GBM may be exacerbated by subclinical CMV infection within these tumors.

**Therapeutic strategies:** Readily available anti-viral agents may have efficacy if viral propagation and gene expression is important in driving the malignant phenotype. Alternatively, immunotherapeutic targeting of CMV-associated tumors may leverage the presence of the virus within tumor cells to elicit specific killing whether the virus serves as a drive of tumor phenotype or merely an opportunistic infection within the tumor microenvironment.
Monocyte harboring CMV

Therapeutic strategies
- CMV-directed immunotherapy
- Anti-viral drugs
- Gene targeting (IL-10, US28)

Uninfected monocyte

CMV

GSCs

vIL-10

Immunosuppressive effects
- Production of vIL-10
- vIL-10 conversion of TAMs
- Downregulation of MHC
- Decreased NK activity
- Increased TGF-beta production

Tumor promoting effects
- Increased cellular invasion
- Reduced p53 & Rb activity
- Increased telomerase
- Increased chemotherapy resistance

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CCR Translations
Is Cytomegalovirus a Therapeutic Target in Glioblastoma?

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