Guest Editorial

Fred Rapp

Cytomegalovirus and Carcinogenesis

HCMV, a virus pathogen familiar to physicians and biomedical scientists since its isolation in 1956 (1, 2), is now a household word. Anyone following the rash of articles on genital herpes and on AIDS sooner or later will learn that HCMV too is a human herpesvirus. It is alarming to discover that HCMV is capable of causing a spectrum of diseases ranging from a mild asymptomatic infection to serious infections resulting in devastating sequelae, such as blindness, deafness, mental retardation, and a host of other life-threatening diseases (3, 4); this includes the ability of HCMV to transfer across the human placenta and to cause severe malformations of the fetus. Thus there is little wonder why HCMV has attracted so much attention in such a short time.

From a biologic perspective, HCMV is an excellent candidate for the role of initiator in carcinogenesis because of its ability to establish persistent infections, to enter a state of latency, and to reactivate. The cellular destruction that occurs as a result of virus latency may be responsible for triggering the succession of events that result in some form of cancer.

One of the challenges of this decade is to identify viral and cellular oncogenes and, ultimately, to control them. Whether scientists will be able to prove with certainty that HCMV is an oncogene involved in the initiation of one or more human cancers is still open for investigation. This editorial addresses the theoretical views supporting the role of HCMV in carcinogenesis.

PROPERTIES OF CMV

HCMV is a member of the family Herpesviridae and is further classified into the subfamily beta-Herpesviridae. Its genome consists of linear, double-stranded DNA with a molecular mass of approximately 150×10^6 daltons (table 1). Beta-herpesviruses share several distinguishing biological characteristics [(5) and table 2]. The most significant is high species specificity; thus their host range in vivo is extremely limited. HCMV has a replication cycle lasting from 36 to 48 hours, which is very long when compared with the 8-hour replicative cycle of HSV. HCMV replicates preferentially in fibroblasts in vitro; however, its in vivo target is often the epithelial cell. On the basis of results of a study of the antigenic relatedness of CMV, there appears to be little cross-reactivity among isolates from different species (6). Although there is extensive cross-reactivity among strains of HCMV, they are antigenically heterogeneous, and this heterogeneity is apparently not the result of clinical disease, geography, or age. No relatedness of HCMV DNA to the DNA of HSV-1, HSV-2, EBV, or nonhuman CMV has been demonstrated by DNA-DNA renaturation kinetics. Results of restriction enzyme digestions and coelectrophoresis of HCMV DNA isolates have revealed that no two isolates have the same HindIII or EcoRI patterns and that each electrophoretic profile is different (7).

Early studies on the replication of HCMV were directed at the development of methods to quantitate the virus and at the effects of drugs on virus synthesis and cytopathology. In 1970, Wentworth and French (8) developed a plaque assay for quantitation of CMV that is still in use today. Besides the difference in lengths of their replicative cycles, another major difference between the cycles of HSV and HCMV is the presence of cytoplasmic dense bodies in HCMV-infected cells, and these dense bodies are almost pathognomonic for HCMV infection (9).

CMV have been found in humans and lower mammalian species, and they can cause an awesome range of diseases. HCMV has a definite preference for replication in human fibroblasts in vitro, although it also is able to replicate in epithelial cells. HCMV infections are characterized by enlarged cells containing intranuclear and cytoplasmic inclusions. Attempts to propagate HCMV in human leukocytes, lymphocyte subpopulations, and hemic cell lines have been unsuccessful (10). However, HCMV and CMV dense bodies can stimulate human peripheral lymphocytes from CMV-positive patients, although there is very little lymphocytic response in seronegative donors (11). Tocci and St. Jeor (12) reported that lymphoblastoid cells of B-

ABBREVIATIONS USED: AIDS = acquired immune deficiency syndrome; CMV = cytomegalovirus; EBV = Epstein-Barr virus; HCMV = human CMV; HSV = herpes simplex virus(es); HSV-1 = HSV type 1; HSV-2 = HSV type 2; KS = Kaposi's sarcoma.

1 Department of Microbiology, Cancer Research Center, The Pennsylvania State University College of Medicine, Hershey, Pa. 17033.

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Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
and T-cell origin are susceptible to HCMV infection; however, few of the cells produced infectious virus.

A number of DNA viruses have known ability to morphologically transform human and nonhuman cells; HCMV is one of these viruses with oncogenic properties (table 3). Albrecht and Rapp (13) first demonstrated the malignant transformation of hamster cells after exposure to UV-irradiated HCMV. They isolated clones that were not contact inhibited and established a continuous cell line from one of the clones. Cells from this line were oncogenic when inoculated into weanling hamsters, and HCMV antigens were detected on the surface and in the cytoplasm of the tumor cells. In 1976, Geder and his colleagues (14) oncogenically transformed human embryo lung cells with HCMV and detected HCMV-specific antigens by immunofluorescence techniques. This same group of investigators also used the HCMV-transformed cells to induce nondifferentiated tumors in weanling athymic nude mice (15). More recently, a transforming sequence in the DNA of HCMV strain AD169 was identified (16). Transfection of NIH 3T3 cells with cloned DNA fragments produced tumors in nude mice. Sequences homologous to the transfecting fragment, however, were not evident in the DNA of the transformed cells (17). Several strains of HCMV have been shown to transform human cells. On the basis of these in vitro studies and work by other investigators, speculation has grown concerning the role that HCMV might have in some types of human neoplasias.

**PERSISTENCE AND TRANSMISSION OF HCMV**

HCMV has the ability to establish persistent infections, to infect cells latently, and to reactivate. Infection with HCMV in humans is probably followed by persistence of the virus in a latent form for life, but reactivation is known to occur in response to an immunologic imbalance in the host. Such imbalance can result from organ and bone marrow transplants, blood transfusions, pregnancy, and immunosuppressive therapies for the treatment of cancers. Two factors are common to cases of reactivation: a) presence of nonhost antigens and b) occurrence of change in the immune system. Although no one is certain of the cell type in which HCMV persists, there is evidence that the virus resides in human lymphocytes (18, 19). It is clear that virus can persist, continue to replicate, and be shed by cells. Latency follows the "persistent" state, whereupon virus is no longer shed by the host, although the genome is still harbored in the body. The quiescent presence of latent HCMV has been demonstrated by the detection of HCMV genetic material, even though whole virus or virus antigens are not detectable.

Transmission of HCMV probably occurs by contact with saliva, urine, and semen since virus is present in these excretions in infected hosts. Acquisition can occur by congenital, natal, and sexual routes and also through blood transfusions and organ and bone marrow transplants. Reactivation of latent virus also can result in transmission of the virus, even from an asymptptomatically infected person. Sexual transmission of HCMV is of particular interest at this time, because most of the neoplasias associated with HCMV are in the genitourinary region.

**ROLE OF HCMV IN CARCINOGENESIS**

Because HCMV shares many properties with HSV and EBV that are characteristic of transforming viruses and because both HSV and EBV are strongly suspected and because both HSV and EBV are strongly suspected of being involved in the etiology of certain human cancers, HCMV is now being given careful scrutiny for its possible role in certain cancers in humans.

**Prostate Cancer**

The first two cancers to be considered involve the genitourinary tract. Adenocarcinoma of the prostate gland is now the fourth most common cause of mortality from cancer in males. HCMV can be harbored in the prostate gland and transmitted sexually, even though individuals can be asymptomatic despite the presence of persistent viruria and viremia. Reports of HCMV in the prostate gland include the detection of herpesvirus antigens in the nuclei of prostatic cancer cells (20) and the presence of HCMV in normal prostate tissue. The presence of human thymidine kinase in prostate cancer cells does not indicate presence of HCMV, even though individuals can be asymptomatic despite the presence of persistent viruria and viremia. Reports of HCMV in the prostate gland include the detection of herpesvirus antigens in the nuclei of prostatic cancer cells (20) and the presence of HCMV in normal prostate tissue.
prostate tissue of a child at autopsy (21). Results of serologic tests showed that a larger percentage of prostate cancer patients had higher antibody titers against HCMV than other groups tested, and that patients with nongenitourinary tumors had lower antibody titers against HCMV (22). These same investigators (23) also reported that a high percentage of patients with adenocarcinoma of the prostate gland and with benign prostatic hyperplasia demonstrated high titers against an HCMV-transformed human cell line. Direct evidence that supports a role for HCMV in prostate cancer is weak, and a cofactor may well be required to initiate this neoplasia.

Cancer of the Cervix

Cervical cancer is still the major cause of death resulting from cancer in women. Although the scientific literature provides many studies involving HSV-2 in the etiology of cervical cancer, HCMV may be an equally strong contender for this dubious distinction, since it is known to reside in semen (24) and is spread sexually. An important study by Pacsa and colleagues (25) reported that in sera of women with cervical atypia, antibodies to HCMV were found more frequently than in sera of women with cervical disorders other than atypia or in sera from healthy controls. However, they also observed higher antibody titers to HSV in the cervical atypia patients than in the matched controls. HCMV has also been isolated from cell cultures established from biopsy specimens of women with advanced cervical cancer (26). It is possible that HCMV may be acting as a cofactor with HSV or another virus in the initiation of cervical cancer, but again, direct evidence is absent.

Adenocarcinoma of the Colon

There are now four reports linking HCMV with bowel disease and adenocarcinoma of the colon (27-30). In addition, cell cultures from 1 patient with regional enteritis demonstrated spontaneous malignant transformation, and HCMV was detected in this patient's cultured cells (31). In some patients with inflammatory bowel disease, a high incidence of antibody to HCMV has been detected, and HCMV has been observed in cultured cells from ulcerative colitis tissue by electron microscopy (31). In their first study Roche and Huang (28) were unable to demonstrate repeatedly the presence of HCMV DNA in normal and abnormal bowels from patients with inflammatory bowel disease, including Crohn's disease and ulcerative colitis. However, 1 year later, they reported the detection of HCMV DNA in 4 of 7 adenocarcinomas of the colon (29) by membrane cRNA-DNA hybridization. Normal and abnormal colon tissues from Crohn's disease patients were negative for HCMV DNA. It was a striking finding, however, when HCMV DNA was detected in patients who had conditions predisposing to adenocarci-
TABLE 5.—Profile of patients with AIDS (as of 7/18/83)

<table>
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<th>Category</th>
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<tr>
<td>1,902 cases reported in the United States</td>
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<tr>
<td>750 deaths</td>
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<tr>
<td>Sexually active homosexual and bisexual men</td>
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<tr>
<td>Abusers of intravenous drugs</td>
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<tr>
<td>Previous venereal diseases</td>
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<tr>
<td>Multiple sex partners</td>
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<tr>
<td>Hemophiliacs</td>
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Different groups have been associated with the disease, and to date most victims of AIDS (table 5) fall within one of the following categories: male homosexuals, hemophiliacs, intravenous drug abusers, and children of mothers with AIDS. These individuals are at high risk for developing AIDS. In male homosexuals with AIDS, a severe acquired cellular immunodeficiency is accompanied by a reversal of the helper-to-suppressor T-cell ratio.

The causative agent of AIDS has not been identified, but because HCMV is immunosuppressive, researchers have investigated its possible connection to AIDS. It is highly possible that following activation of HCMV from a latent state, the immune system is suppressed and Pneumocystis carinii and/or other opportunistic infections flourish (41, 42) and text-fig. 1. It is also possible that defective HCMV is present in the blood, and it is the defective virus that is responsible for immune suppression and the subsequent development of fatal infectious diseases and cancers. HCMV has the ability to destroy cells, to initiate DNA and RNA synthesis, and to transform human cells. These properties suggest that this virus could play a role at multiple points in the development of AIDS and the subsequent sequelae that occur.

SPECSULATION

The role of any virus in human neoplasia has become far more complex than previously thought. The original concept that viruses directly transform cells to cause tumors has to be reevaluated in light of fundamental discoveries in molecular biology. Such observations have revealed that certain retroviruses are capable of transducing cellular genes and that these genes are often capable of transforming cells. The position of other genes near viral promoters also influences the growth of cells. It is still unclear whether cellular genes are required or whether virus genes themselves play a role in the transformation process. It is clear that certain virus genes can transform cells in vitro. Whether these play a role in the oncogenic process in vivo is less clear.

It has been established in many laboratories that inactivated herpesviruses and small pieces of herpesvirus DNA are able to transform cells in culture. It is unclear at this time whether herpesvirus genes are integrated into the host genome. The EBV genome may exist as episomes and integration may not be required for transformation; in that instance, large numbers of virus genomes would be produced and carried to progeny cells during mitosis. However, a recent report (43) suggests that most of the EBV genome is integrated into the chromosomal DNA of cells from a Burkitt’s tumor cell line and into lymphocytes with transformed growth properties in vitro.

It seems likely that viruses with genes capable of converting cells in vitro do play some role in growth regulation in vivo and that under certain circumstances (with low probability), such cells do, in fact, contribute to the oncogenic process under natural conditions. Thus at the present time, it is impossible to state definitively what part HCMV may play in human cancer, but the fact that the virus is able to initiate cellular DNA, RNA, and protein synthesis, that its genome is able to transform cells to cancer in vitro, and that it is a ubiquitous virus found in the human body in many cells strongly merit continued research to explore fully the possibility that this virus may play a role in human neoplasia.

REFERENCES


