Human Herpesvirus 8 Latent-State Gene Expression and Apoptosis in Kaposi’s Sarcoma Lesions

Gary S. Hayward

The evidence that Kaposi’s sarcoma (KS)-associated herpesvirus (also known as human herpesvirus 8 [HHV8]) is the primary etiologic agent of KS in both acquired immunodeficiency syndrome (AIDS) and non-AIDS situations has been accumulating rapidly. Two recent articles, one by Dupin et al. (1) and the other by Sturzl et al. (2), which appears in this issue of the Journal, provide the most compelling evidence yet for the almost universal presence of HHV8 and its latent-state gene products in the spindle-like tumor cells characteristic of late-stage angiogenic nodular KS lesions. Although previously known as only a very rare skin condition occurring primarily in elderly Mediterranean Jewish men (classical KS) and in adolescent males in the malaria belt in central Africa (endemic KS), KS has come to sudden prominence since the early 1980s in the United States as one of the original defining conditions of the AIDS epidemic in young homosexual men. The incidence of KS is now recognized to be increased more than 20,000-fold in homosexual AIDS patients and more than 500-fold in solid organ transplant patients (iatrogenic KS) relative to ethnically matched control subjects, and KS has become the most frequently encountered tumor in some sub-Saharan African countries.

In 1990, Beral et al. (3) predicted that AIDS-associated KS was caused by a second infectious agent transmitted independently of human immunodeficiency virus type 1 (HIV-1), and Chang et al. (4) in 1994 identified a novel herpesvirus genome related to Epstein-Barr virus (EBV) within KS lesions. Subsequently, the 80 or so genes of this virus were mapped and characterized in record time (5–9). Footprints of the virus have been found by DNA polymerase chain reaction (PCR) assays in virtually all KS lesions as well as in several AIDS-associated lymphoid tumors (known as pleural effusion lymphoma [PEL] and multicentric Castleman’s disease), and high-quality serologic reagents are now available to detect infected individuals.

It is not in the best interests of a virus to cause lethal tumors in its natural host, but the evidence from recent strain variability analysis (10) clearly suggests that HHV8 is an ancient human virus and is not a recently acquired pathogen. However, the gammaherpesvirus group to which HHV8 belongs has evolved mechanisms to immortalize the infected cells so that they continue to proliferate while the viruses maintain a long-term quiescent or latent state with minimal viral gene expression to provoke host immune responses. This scenario provides a reservoir for the virus to persist and from where it might also have a route to periodically reactivate and escape to infect a new host. Skepticism has been expressed from some quarters that a ubiquitous lytic herpesvirus could not provoke tumor growth, and it may just be a passenger virus brought to the site accidentally by infiltrating inflammatory cells or that HIV-1 must be the real culprit (11). However, unexpected but important discoveries that HHV8 encodes a series of novel genes (5–9) give credence to the tumorigenic properties of HHV8. The genes include viral homologues of cellular interleukin 6 (IL-6), macrophage inflammatory protein 1 (MIP-1), Bcl-2, and interferon regulatory factors (IRFs) (5–9) as well as a cyclin-D (cyc-D) homologue, an alpha chemokine G-protein-coupled receptor (GCR) encoded by open reading frame (ORF)-74, and tyrosine kinase-signaling membrane proteins encoded by ORF-K1 and ORF-K15. Furthermore, several of these proteins have constitutive in vitro transforming and even angiogenic properties (12–15).

Unlike its cousin EBV, which is ubiquitous in all human populations, HHV8 seropositivity (as a measure of current or previous infection) is rare in the general population (probably no more than 1% in the United States), except in those countries that have relatively high levels of classic or endemic KS. However, HHV8 seropositivity rates in homosexual males are as high as 50%, and they are up to 85% in AIDS patients (16,17). The increased rates of KS in homosexual AIDS patients are probably attributable to a combination of higher rates of infection by HHV8 in that population, as well as to CD4 depletion and immunosuppression and possible direct enhancing effects of HIV-1 itself; in contrast, in sub-Saharan Africa, where HHV8 was already prevalent, the huge increase in KS incidence is attributable to the rapid spread of HIV-1. Infection with HHV8 does not by itself introduce a high risk of contracting KS, although it is now almost certain that no cases of KS occur without the presence of HHV8 as the primary triggering etiologic agent. However, for those AIDS patients infected with both viruses, the chance of developing KS within 10 years may be as high as 50%.

Obviously, for a virus of this type that cannot yet be grown efficiently in cell culture and is very unlikely to be transmissible into animal models, classical Koch’s postulates cannot be fulfilled. However, apparently infectious virus can be recovered from filtered supernatants derived from TPA (phorbol 12-myristate 13-acetate)-treated PEL cell lines and gives rise to latently infected, proliferating, spindle-shaped cells in primary human endothelial cell cultures (18,19). The PEL cell lines themselves and even NIH/3T3 cells transformed in vitro by the vGCR gene of the virus give rise to highly angiogenic tumors in nude mice (12,13). One drawback has been that spindle cell lines established from KS tumors themselves fail to retain the virus. Perhaps the virus reactivates and kills those cells that carry it, or the surviving cells that grow out have mutations that bypass the need for the virus to maintain the production of vascular endo-

Correspondence to: Gary S. Hayward, Ph.D., Molecular Virology Laboratories, Departments of Oncology and of Pharmacology and Molecular Sciences, The Johns Hopkins School of Medicine, 725 N. Wolfe St., WBSB 317, Baltimore, MD 21205 (e-mail: ghayward@jhmi.edu).

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that HHV8 also encodes a lytic cycle-deregulated anti-apoptotic Bcl-2 gene (35).

The study by Dupin et al. (1) also shows for the first time convincing evidence for LANA expression in some immature vessel-associated endothelial cells that also express VEGFR3 in most early patch and plaque KS samples. Early-stage lesions often have relatively few spindle cells, and Sturzl et al. (2) failed to detect mRNAs for vFLIP, v-cyc-D, or LANA in them, but they did find many apoptotic cells. These apparent differences emphasize an important cautionary point that levels of mRNA do not necessarily match levels of protein expression: In this case, the LANA protein can be detected by immunohistochemistry, whereas the levels of LANA mRNA appear to be below the threshold for detection by in situ hybridization. Conversely, although vFLIP mRNA can be detected, there is as yet no evidence that the corresponding vFLIP protein is made, which is an especially important point because it represents only the second or third downstream ORF in these transcripts. Some evidence that the v-cyc-D protein is expressed in at least some KS samples has been presented (36), but much more is needed to address the levels, timing, and frequency of both v-cyc-D and vFLIP expression and to connect these effects to proliferation and neoplasia in KS spindle cells.

Despite encoding all of these viral proteins that have obvious potential to contribute to KS pathogenesis, only a small number are candidates for providing direct effects as latency products in all infected spindle cells. Besides LANA (possibly functionally analogous to EBV nuclear antigen, EBNA1) and the v-cyc-D/vFLIP, only ORF-K15 (structurally resembling LMP2 [latent membrane protein 2] of EBV) and perhaps T0.7/K12/kaposin have been identified as solid candidates for latency gene products. Of course, there is always the caveat that lytic cycle gene products may be expressed in just a few partially or abortively induced cells, which could function similarly to the relatively rare EBV-infected Reed-Sternberg tumor cells in Hodgkin’s lymphoma (37). In fact, there is also evidence that the early lytic cycle vIL6, vIRF, chemokines, and vGCR proteins may be expressed in a small fraction of lymphoid tumor cells and possibly also in late-stage KS, suggesting that they may produce paracrine effects that contribute to the spindle cell proliferation or angiogenesis as well as to avoidance of immune responses or perhaps even contribute to reducing the likelihood of an otherwise limited skin neoplasia progressing to uncontrolled malignancy.

Criticisms that KS may not be a true malignancy (in the sense of having irreversible genetic changes) but is rather a polyclonal or mixed polyclonal/monoclonal hyperplasia are not very germane to the issue of whether HHV8 drives it, nor are they of much comfort to a patient with aggressive disease that has disseminated to internal organs. KS lesions are known to undergo spontaneous remissions, especially with cessation of immunotherapy in iatrogenic cases or sometimes after therapeutic interventions with interferon alfa or chemotherapy, and there are anecdotal reports of considerable improvement under HAART (highly active anti-retroviral therapy) regimens, but not every patient responds and it is not known whether these are cures or just temporary effects. Hopefully, these new findings will lead to intensification of efforts to understand the basic fundamentals of this intriguing disease process and continued searching and testing for suitable antiviral and antineoplastic agents that can control KS, especially in the developing countries of Africa.
REFERENCES


