Kaposi's Sarcoma-Associated Herpesvirus

Recent findings on the etiology and pathogenesis of Kaposi's sarcoma (KS) and on the association of a novel herpesvirus with KS were discussed at the San Francisco conference by Donald E. Ganem, MD, from the University of California San Francisco.

Characteristics of Kaposi's Sarcoma

Kaposi's sarcoma (KS) is an atypical tumor; indeed, KS should probably not be thought of in the same way as most cancers. Whereas most tumors derive from the clonal outgrowth of a single malignant cell, KS tumors do not appear to evolve in this manner. The exact events involved in their formation remain unclear. In advanced lesions, the predominant cell is the spindle cell. It is now generally believed that spindle cells are of endothelial origin, although precisely where in the endothelial cell lineage these cells originate remains the subject of investigation and debate. Spindle cells lack many of the markers of mature endothelial cells, with a very small percentage (2% to 5%) having the canonical factor VIII marker. Other evidence suggests that spindle cells are heterogeneous: 80% to 85% of these cells are positive for the endothelial cell marker CD34 and a similar proportion is positive for the CD36 marker. However, 5% of spindle cells are positive for smooth muscle actin. Thus it has been proposed that spindle cells may be derived from primitive, bipotential mesenchymal cells that can become either vascular smooth-muscle cells or vascular endothelial cells.

The complexity of KS lesions is underscored by the fact that they contain many infiltrating plasma cells, lymphocytes, and other inflammatory cells, as well as spindle cells. In addition, KS lesions are characterized by a profusion of aberrant slit-like neovascular spaces with extravasated red blood cells. Furthermore, KS often presents in multicentric fashion, with lesions appearing simultaneously at different sites. Kaposi's sarcoma is also characterized by an unusual propensity for regression or exacerbation, which is again atypical of most cancers. Lesions have been found to regress with reduced immunosuppression in posttransplantation patients and in patients treated with protease inhibitors for HIV disease. Tumors in KS have been reported to progress very rapidly following severe episodes of sepsis or Pneumocystis carinii pneumonia (PCP) in patients with HIV disease.

Early studies of the pathogenesis of KS showed that cultured spindle cell require growth factors for their proliferation and that they produce a host of growth factors and factors with some angiogenic potential. Interestingly, cultured human spindle cells are nontumorigenic in nude mice: complete involution of these cells occurs within 1 to 2 weeks. However, prior to their involution, these cells lead to the development of lesions consisting of aberrant blood vessels and infiltrating inflammatory cells of murine origin. These neoangiogenic, KS-like lesions resolve following the involution of the human spindle cells. These findings led to the currently favored model of histogenesis in which the proliferation of spindle cells is accompanied by the production of growth and angiogenic factors, which in turn, results in the formation of new blood vessels and the presence of plasma cells and lymphocytes.

Role of HIV in KS Pathogenesis

What triggers spindle cell proliferation? Early attention regarding this issue focused on the role of HIV. HIV infection is associated with a 20,000-fold increased risk for KS, and models proposed to explain the role of HIV in KS have greatly influenced studies of KS pathogenesis. In one proposed model, HIV-infected cells produce cytokines and other growth factors (e.g., basic fibroblast growth factor and oncostatin M) that trigger the proliferation of spindle cells. The researchers who formulated this model showed that HIV-infected cells also release small quantities of Tat protein, which can bind to cell-surface receptors of the integrin family and stimulate spindle-cell proliferation.

One problem with a model that requires only the effects of HIV infection to induce KS is that KS tracks poorly with HIV infection within the cohort of patients with AIDS. A number of investigations have shown that the prevalence of KS varies in patients with AIDS: 15% to 35% in male homosexuals, 1% to 3% in transfusion recipients and in persons with hemophilia, and less than 1% in children with vertically transmitted HIV disease. This variability in the prevalence of KS suggests the presence of an etiologic cofactor other than HIV, as does the fact that KS occurs in HIV-negative populations, including elderly Mediterranean men, residents of certain areas in Africa, and transplant recipients. The pattern of KS cases in AIDS is most consistent with the cofactor being sexually transmissible.

Association of Human Herpesvirus 8 with KS

Given the epidemiology of KS, a number of research groups began to search for a viral etiologic agent in KS.
The approach that succeeded was to identify viral DNA in KS tissue that was not present in non-KS tissue. Two small exogenous DNA fragments—300 kilobases (kb) and 600 kb in length, respectively—were found to track closely with KS. In one series, these viral markers were found in more than 95% of AIDS patients with KS, in 10% of lymph node samples from AIDS patients without KS, and in no tissue samples from subjects without HIV infection. The DNA sequencing revealed that these DNAs derive from a novel herpesvirus, termed KS-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8). These DNA sequencing studies have shown that HHV8 is related to the gamma herpesviruses, or lymphotropic herpesviruses, of which the Epstein-Barr virus (EBV) is the best-known agent in humans. However, HHV8 is much more closely related to the simian virus Herpesvirus saimiri than to EBV. (The typical host of H saimiri is the squirrel monkey in which it causes asymptomatic infection. In the related owl monkey, however, this virus causes an aggressive T-cell lymphoma.) It has subsequently been shown that the HHV8 DNA sequences originally found in AIDS-KS tissues are also present in KS tissues from patients without HIV infection.

The association of HHV8 with KS was further supported by findings in a study that used a polymerase chain reaction (PCR) assay for viral DNA in peripheral blood mononuclear cells (PBMCs). This assay is positive for HHV8 in approximately half of patients with KS. Whitby and colleagues tested stored samples of PBMCs from HIV-positive patients who did not have clinical KS at the time of sampling to examine the relationship between the presence of viral DNA and subsequent development of KS. In a cohort of 143 AIDS patients without KS, 11 subjects had PBMC samples that were positive for HHV8. Of these 11, 55% subsequently developed KS. Of the 132 AIDS patients whose PBMC samples were negative for HHV8, 9% subsequently developed KS. These findings indicate that HHV8 infection precedes the clinical onset of KS, suggesting that HHV8 infection is not a superinfection of the KS lesion.

Subsequent cloning of the HHV8 genome has shown that it is 160 kb in length. Dr Ganem and colleagues are currently attempting to identify the genes produced in the KS tumor as a means of better understanding the pathogenesis of KS lesions. Previous studies of cultured spindle-cell lines indicated that the viral genome was not present in spindle cells, and some investigators speculated that the lymphoid cells in KS were the likely target of HHV8 infection. However, Dr Ganem and colleagues as well as other researchers have found that spindle cells are in fact the in vivo target of HHV8 infection. Probes were developed by identifying and cloning the most abundant viral messenger RNA (mRNA) fragments in KS and using them in in situ hybridization studies. These investigations showed that there are at least two different transcriptional programs occurring in KS. Most spindle cells (80% to 90%) are latently infected. A minority of these cells (1% to 3%) seem to be lytically infected by HHV8, and, therefore, are likely to be producing virus. The latently infected cells are only expressing a small subset of viral genes, and studies are ongoing to identify all of these genes. The role of the lytically infected subset of cells is at present unclear.

**Initial Seroprevalence Findings**

Dr Ganem and colleagues have recently developed a system for viral growth in cell culture that will allow a better understanding of HHV8 replication and should facilitate studies of the pathogenesis and epidemiology of KS. Human herpesvirus 8 has been found in AIDS patients with rare B-cell lymphomas (i.e., body-cavity based lymphomas). These lymphomas do not involve bulky lymphadenopathy; instead they are characterized by malignant effusion usually of the pleural space or peritoneum consisting of a monoclonal population of malignant B-cells. It was initially believed that HHV8 was always present with EBV in these lymphomas; however, recent investigations have shown that HHV8 can be present without EBV in such tumors. A cell line for HHV8 culture has been developed from one of these cases of lymphoma in which EBV was not present. The cells, which replicate in vivo as an ascites tumor, grow readily in culture medium.

Using this cell line, Dr Ganem and colleagues have developed an immunofluorescence test for HHV8 antibodies that is not complicated by the presence of EBV antibodies, which are nearly ubiquitous in the population. This assay, which in its current form has been found to detect HHV8 antibody in approximately 85% of AIDS patients with KS, has been used in initial seroprevalence studies. In a cohort of 150 blood donors who were negative for HIV and for hepatitis B and C viruses, Dr Ganem and colleagues found that the prevalence of HHV8 antibody was approximately 1% to 2%. In a group of 120 HIV-negative men with positive Venereal Disease Research Laboratories (VDRL) test results who presented to a sexually trans-
mitted disease (STD) clinic, 8% were found to be positive for HHV8 antibody. In still another cohort of 125 HIV-seropositive men, most of whom were gay, Dr. Ganem and colleagues found that 31% were positive for HHV8 antibody. In contrast, only 2% to 3% of 300 HIV-positive persons with hemophilia were found to be HHV8 seropositive. These findings suggest that HHV8 infection is not ubiquitous in the general population (as are many other herpesviruses) and that there is a strong association between markers of sexual activity and acquiring HHV8 infection. In addition, they show a striking link between HHV8 infection and risk of KS. Taken together, the evidence strongly suggests that HHV8 is the sexually transmitted cofactor predicted by the epidemiology of KS.

Implications for Prevention and Treatment of KS

What are the implications of this research for the prevention and treatment of KS? Clearly, if further evidence confirms that HHV8 infection predisposes a patient to the development of KS, strategies aimed at preventing this infection will assume a high priority. Investigators need to determine which specific practices confer the greatest risk of transmission. With such detailed information not yet available, it seems prudent to add HHV8 to the list of reasons for adhering to current safer-sex guidelines. Development of a vaccine for HHV8 infection will also need to be initiated in parallel. A successful vaccine has been developed for at least one herpesvirus infection (for the varicella-zoster herpesvirus, which causes chickenpox and shingles); thus, success is possible in principle. In practice, however, there are many potential obstacles and development of a vaccine for HHV8 infection is not likely within the next ten years.

An important question concerns the role of antiviral drugs. Since most KS spindle cells are latently infected, it is not likely that conventional antiviral therapy will have a dramatic effect on established KS tumors, even though available antiviral drugs target lytic viral growth. However, it is possible that such therapy could have an impact on the natural history of HHV8 infection in asymptomatic HHV8-seropositive patients. For example, reducing the number of infectious virions may reduce viral spread to susceptible spindle-cell targets. Over time, sustained therapy may reduce the risk of subsequent development of KS. However, it is to be emphasized that at present there is no solid evidence for such a scenario. Moreover, such a regimen would require an orally-active drug with an exceptionally favorable side effect profile, since therapy would presumably have to be protracted. None of the currently available drugs that are active against HHV8 meet this standard.

Donald E. Ganem is Professor of Microbiology/Immunology and Investigator, Howard Hughes Medical Institute, University of California San Francisco.

Suggested Readings


