Kaposi sarcoma–associated herpesvirus (KSHV) is a recently discovered and characterized member of the herpesvirus family. It is one of a few viruses proved to be associated with tumorigenesis in humans. Its causal association with 4 clinical and epidemiologic variants of Kaposi sarcoma (classic, endemic, iatrogenic, and acquired immunodeficiency virus–associated) as well as with several lymphoproliferative disorders (notably primary effusion lymphoma and multicentric Castleman disease) is reviewed critically. Issues related to the epidemiology, transmission, and molecular and serologic diagnosis are discussed. Several intriguing oncogenic mechanisms of KSHV infection have been identified. These are often dependent on the interaction of KSHV with other viruses, such as human immunodeficiency virus, Epstein-Barr virus, or both. However, important problems remain and once resolved will substantially enhance our understanding of oncogenesis in general and viral-induced oncogenesis in particular. This may also translate into improved treatment and perhaps prevention of this common and intriguing viral infection.

In 1994, Chang et al identified the presence of DNA fragments of a novel herpesvirus in tumor tissue specimens from a patient with acquired immunodeficiency syndrome (AIDS)–associated Kaposi sarcoma (KS). Within 2 years of its discovery, the 165-kilobase genome of the KS-associated herpesvirus (KSHV; also designated human herpesvirus 8) was fully sequenced. Soon after, the newly discovered virus was established as the primary causative factor in all types of KS, as first described in 1872 by Hungarian dermatologist Moritz Kaposi. More recently, KSHV was also linked with the pathogenesis of several lymphoproliferative disorders, and the importance of immunosuppression in the pathogenesis became recognized. Extensive virologic and epidemiologic research has not only resulted in further elucidation of molecular structure, serologic profile, and mode of viral transmission but also has provided unique insights into the mechanisms of cancer in general and virus-associated neoplasia in particular.

KSHV VIROLOGIC ASPECTS

Virus Epidemiology

Insight into the epidemiology of KS, the most common KSHV-associated disease, provided valuable clues to the biology of the virus and its transmission. Four clinical and epidemiologic variants of KS have been recognized: classic, endemic (African), transplantation associated (iatrogenic), and epidemic (AIDS associated). Although all variants share KSHV-mediated pathogenesis and viral DNA can be detected in virtually all lesions of KS, several different characteristics should be recognized. Classic KS occurs predominantly in elderly men of Mediterranean or Eastern European descent and evolves slowly, sometimes over decades, starting usually on the feet and involving primarily the skin only. Endemic KS is the most frequently occurring tumor in men in certain Central African countries, whereas since the advent of AIDS, it has become more common in both sexes in Africa, with a significant lowering of the male-to-female ratio from 19:1 to 1.7:1, especially in East Africa. It takes a form similar to classic KS in human immunodeficiency virus (HIV)–negative adults but affects children as well in a progressive lymphadenopathic form that is often rapidly fatal. Early-life KS is extremely unusual in non-African populations. In addition, KS occurs after immunosuppressive therapy, especially in the setting of organ transplantation. This form tends to be clinically aggressive, underscoring the impor-
tance of the immune system in the outcome of KSHV infection. Indeed, discontinuation of immunosuppressive therapy has been associated with clinical remission. Up to 5% of transplant recipients in high prevalence areas but considerably fewer patients in areas with low prevalence of KSHV are affected. Iatrogenic KS results mostly from reactivation of preexisting infection; however, virus transmission from the transplanted organ has also been shown. AIDS-associated KS is the most prevalent form of KS today and the most common AIDS-associated cancer in the United States. It affects homosexual males (a 50% lifetime risk of developing KS before the advent of highly active antiretroviral therapy [HAART]) much more than other HIV-infected individuals with a similar degree of immunosuppression. These patients exhibit a more widespread cutaneous, lymphatic, and oral involvement and may develop a frequently fatal visceral KS. The severity and progression of KS in AIDS correlate with the viral load and are inversely related to the CD4+ T-cell count. In general, the incidence of KS among AIDS patients has substantially declined after the introduction of HAART. Furthermore, KS in patients with AIDS may respond to HAART.

Primary or acute infection with KSHV has been poorly defined. To our knowledge, there is no description of characteristic symptoms that accompany primary infection in healthy individuals. A single case of an AIDS patient who seroconverted to KSHV after a transient episode of fever, lymphadenopathy, and arthralgias has been reported, as well as 2 cases in kidney transplant recipients who developed fever, splenomegaly, cytopenia, and bone marrow failure coinciding with KSHV viremia. The KSHV seroconversion in HIV patients seems to follow a brief, low-grade viremia.

Unlike other human herpesviruses, KSHV is not ubiquitous. Seropositivity rates for KSHV show remarkable racial and geographic variations. Infection rates are less than 3% in the United States and most European countries. Infection rates are up to 25% in Mediterranean regions such as southern Italy and may be substantially more than 50% in Uganda and other Central African countries. Thus, most KSHV infections appear to be asymptomatic, and most infected individuals will not develop virus-associated disease. However, untreated HIV patients who acquire KSHV infection after HIV seroconversion are highly likely to develop KS, possibly because of a synergistic effect of the 2 viruses. Specifically, Tat protein of HIV-1 has been shown to act as an angiogenic factor and as a stimulator of KSHV replication. Therefore, HIV-1 infected individuals may be at high risk of developing KS not only because of the acquired deficiency of cellular immunity but also because of specific interactions between both viral proteins.

Generally, the seroprevalence of KSHV correlates with the incidence of KS. Southern Italy’s tripled seropositivity rate compared with that in northern regions mirrors KS incidence. Nevertheless, KS seems unexpectedly uncommon in some African populations despite high KSHV seroprevalence. In these populations, other cofactors specifically associated with HIV-1 rather than HIV-2 infection may be implicated in the development of KS. This suggests that unknown cofactors may modify the clinical expression of viral infection. Genetic factors, concurrent infectious and environmental pathogens, and possibly molecular variants of KSHV that may differ in pathogenicity are probably important for establishment of KSHV-associated diseases.

**Virus Transmission**

Sexual transmission of KSHV is well established, particularly through male homosexual contact. Thus, the prevalence of infection is associated with the number of homosexual partners and correlates with a history of sexually transmitted diseases. The risk factors for KSHV acquisition in heterosexual people are less clear. Although a reported increase in virus prevalence after puberty in developed countries further supports transmission by sexual contact, prepubertal virus prevalence observed in developed countries is lower. Although transmission of KSHV was recently suggested, and family members of KS patients have a 3-fold higher KSHV seroprevalence rate compared with the general population. Although KSHV can probably be transmitted by organ transplantation, evidence supporting blood-borne transmission is still relatively poor. Even among HIV-positive patients, the prevalence of KS in hemophilia patients, intravenous drug users, and transfusion recipients is low, and recipients of bone marrow transplants also show no increased rate of infection. Because virus sequences have been detected in peripheral blood mononuclear cells and plasma of KS patients, large-scale screening of blood donors and parallel analysis of recipients in areas endemic for KSHV may clarify the actual effect of blood-borne transmission.

**Molecular and Serologic Diagnosis**

The first diagnostic tool in the detection of KSHV infections was based on the use of polymerase chain reaction
(PCR) to amplify viral DNA\(^1\) (Figure 1). Southern blot hybridization is being used as an additional confirmatory test. Each detects viral DNA in virtually all lesions of KS, providing one of many other clues and firmly establishing causal relationship.\(^1\) The PCR studies were also applied to determine and quantify the presence of KSHV DNA in tissues and bodily fluids. Tissue localization of the virus can be further studied by immunohistochemical and in situ hybridization techniques. At the same time genetic variability of the virus is being increasingly recognized, although its clinical importance remains uncertain.\(^1\) These types of studies suggest that KSHV is an ancient human virus principally transmitted in a familial mode with low recombination rates.\(^1\)

Serologic assays are currently used in research laboratories to diagnose infection. Antibody response persists for the lifetime of the patient and may therefore be used to establish the prevalence of infection and evaluate risk factors for transmission. Serologic assessment may also be important for surveillance and prevention of KSHV-related diseases, predominantly in organ transplantation in areas of high prevalence and in HIV-infected patients. Various formats of diagnostic serologic tests already exist; however, only a few are commercially available. Initial widely used serologic tests use immunofluorescence assays to detect antibodies to latent or lytic viral antigens expressed in KSHV-infected cell lines derived from patients with primary effusion lymphoma\(^35,37\) (Figure 1). However, interobserver differences may produce a variation in the results. Several enzyme-linked immunosorbent assays (ELISAs) were also established by using purified recombinant KSHV immunogenic lytic antigens (open reading frame [ORF] 65, 26, K8.1) or latent antigens (ORF 73, K12).\(^38,39\) Additional ELISAs with one peptide selected from an immunogenic viral protein, a mixture of peptides, or a whole virus were developed.\(^38,39\) Immunoblotting techniques have been applied for most of these antigens.

A sensitive and specific “gold standard” method for identifying infected individuals has not yet been established. Several large studies\(^15,38,39\) compared the performance of the various serologic assays. The concordance for KS sera is relatively high, whereas it decreases in healthy
individuals, African patients, and KS-free HIV-infected patients. Because certain serologic methods couple increased sensitivity with a decreased specificity and vice versa, a higher predictive value is achievable with combinations, preferably with antigens representing both lytic and latent virus proteins.38 Interestingly, recent data suggest that a combined increase in PCR and antibody signals may predict KS onset.40 The development of a universal algorithm for a routine serologic diagnosis is pending and remains a highly important goal.

Oncogenic Mechanisms

Together with human T-cell lymphotropic virus 1, hepatitis C virus, human papillomavirus, and Epstein-Barr virus (EBV), KSHV is capable of inducing malignant tumors in humans. Like other oncogenic viruses, KSHV has evolved various mechanisms for immortalizing and transforming cells (Figure 2). Its viral genome contains several genes that are homologous to proto-oncogenes, cellular genes capable of inducing malignant tumors.44

Thus, a viral homologue of cyclin D can inhibit the retinoblastoma tumor-suppressor protein.42-44 Furthermore, the cellular inhibitors of this pathway (p16, p21, and p27) do not inhibit the virally encoded cyclin D protein.44 Concomitantly, other viral genes, such as the latency-associated nuclear antigens 1 and 2 and viral interferon regulatory factors, interfere with the p53 tumor suppressor pathways, which regulate cellular senescence and apoptosis.54-56 Certain viral genes homologous to cellular antiapoptotic genes may facilitate malignant transformation.49-51 In addition, viral chemokines and cytokines, such as interleukin 6, have numerous tumor-facilitating effects, including an effect on B cells and a possible induction of angiogenesis, which may contribute to the pathogenesis of the KS lesion.52 A transformed phenotype was obtained when the virus G protein–coupled receptor homologue protein or the unique Kaposin protein was overexpressed in vitro.53-56 No less important is the recent recognition of some of the mechanisms by which KSHV evades recognition and attack by the host’s cytotoxic T lymphocytes. Apparently, KSHV encodes 2 unique gene products that down-regulate class I major histocompatibility complex expression.57-61

However, despite all these rapid advances in understanding the molecular biology of KSHV, crucial questions remain. Although KSHV encodes an arsenal of potential oncogenes, tumor formation is observed rarely. In fact, most infections are classified as persistent-latent types with no known clinical features. Nonetheless, primary endothelial cells, infected by KSHV, acquired a transformed phenotype.62 Distinct patterns of expression of viral genes have been noted in different KSHV-associated diseases, possibly providing a clue for the wide clinical spectrum.63

KSHV-ASSOCIATED DISEASES

Kaposi Sarcoma

The lesions of KS have distinctive distribution, morphologic features, and histologic findings. Multifocal, usually painless skin involvement is typical, with the gradual appearance of reddish purple macules or patches or purple nodules on the legs. Lymphadenopathy is common with the more aggressive forms, such as HIV-associated KS. In such patients, upper body location of the lesions, mucous membrane involvement (particularly oropharyngeal lesions), and visceral involvement (mostly gastrointestinal or pulmonary) may be prominent. The histologic features are uniform, showing spindle-shaped tumor cells with extravasated red blood cells and hemosiderin in slits between irregular vascular spaces.6 Distinguishing between the classic, African, iatrogenic, and AIDS-associated variants of KS is therefore clinical and has important implications for prognosis and therapy.
The main obstacles in the treatment of KS are its multifocal nature, tendency to recur, and dependency on the host's immunodeficiency state. Single lesions can be treated by surgical excision. Other effective local treatments are limited doses of radiation (8-12 Gy) or intralungal injection of interferon alfa. Patients with extensive, recurrent, or visceral KS pose a considerable therapeutic challenge. Instituting HAART in patients with AIDS-associated KS and modifying or discontinuing immunosuppressive treatment in recipients of organ transplants when feasible are highly important. Systemic chemotherapy either with a single-agent regimen (eg, vinblastine, vincristine, etoposide or bleomycin) or combination chemotherapy (eg, Adriamycin, bleomycin, and vincristine) may be used, with response rates varying from 50% to 88%. Liposomal doxorubicin has improved pharmacokinetics and enhanced accumulation in tumor tissue, which makes it a promising first-line therapy in patients with advanced disease. Paclitaxel is another good choice, providing a major response rate of approximately 60% for a median of 10.7 months, even in patients in whom prior chemotherapy failed. Several experimental agents are being studied. Thalidomide inhibits angiogenesis, retinoic acids block 2 major autocrine growth factors in KS (oncostatin M and tumor necrosis factor α), and human chorionic gonadotropin treatment is based on the increased susceptibility to KS in men and the failure of KS cell lines to grow in pregnant mice. These and other pathogenesis-based therapies may hold hope in the future treatment of patients with KS.

Primary Effusion Lymphoma

In 1989, a new peculiar and rare form of lymphoma was described in HIV-positive patients. This form of lymphoma, originally named body cavity–based lymphoma, was found to be ubiquitously associated with KSHV. It was later designated primary effusion lymphoma (PEL) and established as a distinct clinical entity. Most cases occur in HIV-infected individuals. Clinically, it presents as lymphomatous growth in the liquid phase, affecting pleura, peritoneum, and pericardium either singly or in any combination. Typically, there are no lymphomatous masses, although some patients (15% of HIV-associated cases) present with extranodal tumor masses, whereas effusions develop later in half of the patients.

Most tumor cells are immunoblast-like cells, but some large anaplastic lymphocytes with numerous or multilobulated nuclei resembling Reed-Sternberg cells may also be present. Phenotyping may be difficult because the cells characteristically lack the lineage-restricted markers of both T and B lymphocytes. Activation antigens, such as HLA-DR, CD30, CD38, and CD71, are present in most cases tested. Clonal immunoglobulin gene rearrangement found in all cases studied firmly establishes PEL as a B-cell neoplasm. In almost all the HIV-associated PEL cases, clonal genetic material of EBV is present, but infection with KSHV alone is sufficient for PEL. The precise mechanisms of the interaction among KSHV, EBV, and HIV in the pathogenesis of PEL are still unclear. Interestingly, a small number of patients with PEL who were HIV negative have been identified. These patients appear to have unique epidemiologic, virologic, and clinical features, suggesting that PEL in HIV-negative patients may be a distinct entity (Table 1).

Table 1. KSHV-Associated Primary Effusion Lymphoma: Comparison of HIV-Negative and HIV-Positive Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV positive</th>
<th>HIV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>Uncommon</td>
<td>12 cases</td>
</tr>
<tr>
<td>Histologic type</td>
<td>Large B cell</td>
<td>Large B cell</td>
</tr>
<tr>
<td>Age</td>
<td>Young adults</td>
<td>Mostly elderly</td>
</tr>
<tr>
<td></td>
<td>(25-45 y)</td>
<td>(78-101 y)</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M:F = 5:1</td>
</tr>
<tr>
<td>Homosexual males</td>
<td>All patients</td>
<td>None recognized</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td>Variable</td>
<td>Mostly Mediterranean</td>
</tr>
<tr>
<td>EBV status</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Treatment response</td>
<td>Not established</td>
<td>Chemotherapy?†</td>
</tr>
<tr>
<td>Clinical outcome</td>
<td>Fatal</td>
<td>†</td>
</tr>
</tbody>
</table>

*Human immunodeficiency virus (HIV)–negative patients tend to be elderly men of Mediterranean descent (resembling the population susceptible to classic Kaposi sarcoma), and, unlike the HIV-positive cases, their tumor cells do not contain Epstein-Barr virus (EBV) sequences, providing further evidence for an EBV-independent oncogenic potential of Kaposi sarcoma–associated herpesvirus (KSHV). The mechanisms that drive the malignant process toward either primary effusion lymphoma or Kaposi sarcoma in similar populations thus far remain enigmatic. Reprinted with permission from Klepfish et al.

†Cannot be determined but may respond to chemotherapy.
Multicentric Castleman Disease

Castleman disease (angiofollicular lymphoid hyperplasia or giant lymph node hyperplasia) is a rare, nonmalignant, usually polyclonal form of lymphadenopathy. Three histologic types have been identified: hyaline-vascular, plasma cell, and intermediate. The originally described localized Castleman disease is usually of hyaline-vascular histologic type, is located in the mediastinum in 70% of patients, and can usually be cured by surgical excision. Later, a systemic variant, multicentric Castleman disease (MCD), usually of plasma cell histologic type, was described. It has an aggressive, often fatal clinical course and usually presents with multifocal lymphadenopathy and a variety of systemic symptoms, such as fever, rash, cytopenia, and hypergammaglobulinemia. An association with POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal component, and skin changes) has been described. The disease is common among AIDS patients. The KSHV DNA sequences were found in all HIV-related cases of MCD in contrast to less than half of HIV-negative cases. Moreover, KSHV viral load in the peripheral blood of MCD patients tends to correlate with the severity of symptoms, worse prognosis, and exacerbations. Interleukin 6, a cytokine encoded by KSHV, and other viral proteins were detected in lymphoid cells and endothelial cells of patients with MCD. In addition, patients with MCD may develop other KSHV-associated diseases: PEL, KS, or both. Non-Hodgkin lymphoma has also been reported to develop in HIV-positive and HIV-negative MCD patients.

No consensus exists about the optimal treatment of MCD, but alkylating agents, vinca alkaloids, and glucocorticosteroids have apparent activity in MCD treatment. A response to interferon alfa has also been reported.

Anaplastic Large Cell Lymphoma

Three AIDS patients with solid extranodal (lung and skin) lymphoma, exhibiting anaplastic large cell morphologic features and expressing CD30, were recently described. In all cases, KSHV was detected by PCR, and in 2 of 3, EBV DNA sequences were also detected by in situ hybridization, suggesting a novel subtype of KSHV-associated lymphoma.

Multiple Myeloma

In 1997, the presence of KSHV was shown in nonmalignant dendritic cells from long-term bone marrow cultures of multiple myeloma patients. The same group soon confirmed this finding on fresh bone marrow samples of most myeloma patients tested and one fourth to one third of patients with monoclonal gammopathy of unknown significance, a percentage similar to the proportion of patients with monoclonal gammopathy of unknown significance whose disease progresses to myeloma throughout the years. The pathogenetic relevance of the findings was further supported by the demonstration of the ability of viral interleukin 6 to support growth of myeloma cells in culture. Several other reports have provided at least partial support for the relevance of KSHV in multiple myeloma. However, most studies performed by several groups of investigators showed no evidence of KSHV association with multiple myeloma, and currently the association remains highly debatable.

UNRESOLVED ISSUES AND FUTURE DIRECTIONS

Major advances have marked the short history of KSHV identification and research. However, crucial and intriguing questions still remain. Like other herpesviruses, most primary KSHV infections are followed by a lifetime latent phase characterized by low-grade virus replication and a potential for reactivation. The site of latency and its possible effect are still unknown. The latency is probably associated with the hematopoietic system. The mechanisms by which the virus escapes elimination by the host’s immune system and the events leading to reactivation are incompletely understood. Although the role of host immunodeficiency in KSHV activation and pathogenicity is well established, additional important factors in the transition between latent asymptomatic viral carriage and the development of KSHV-associated diseases remain to be elucidated. The repertoire of KSHV-associated diseases may not yet be complete, and future research is also needed to determine the full effect of viral coinfections.

An optimal prevention of KSHV infection and diseases can be achieved only with an effective vaccine not yet available. Whether serologic and molecular screening for the virus to prevent the transmission and the resulting morbidity in high-risk persons (eg, organ transplant recipients, HIV-infected sexual partners, blood donors, and candidates for immunosuppressive therapy in endemic areas) is justified has to be evaluated. Laboratory studies show that KSHV is sensitive to ganciclovir, foscarnet, and cidofovir, suggesting that chemoprevention in KSHV carriers exposed to immunosuppression may be feasible. However, since these compounds inhibit viral DNA replication but have no effect on the expression of viral latent genes, they may be applied only as preemptive agents or during active disease. In fact, treatment of AIDS patients with oral or intravenous ganciclovir reduced the risk of KS, whereas intravenous ganciclovir and foscarnet therapy had no effect on KSHV DNA load in 7 KS patients. Therefore, more clinical studies are necessary before any conclusions can be made.
Finally, the short time (less than a decade) that has elapsed from the discovery of KSHV as a novel herpesvirus to the deciphering of its most important disease associations and oncogenic mechanisms is striking evidence of the extent of the recent advances in scientific investigation and knowledge. Improved understanding of the crucial issues, which remain unresolved, is likely in the foreseeable future, and we hope this translates into improved clinical outcomes and possibly prevention.

REFERENCES