The Kaposi’s sarcoma-associated herpesvirus (human herpesvirus-8) in Kaposi’s sarcoma, malignant lymphoma, and other diseases

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Summary

Background: Two novel nonhuman DNA fragments were discovered in an AIDS-related Kaposi’s sarcoma (KS) lesion using representational difference analysis.

Design: These sequences belong to a previously unidentified gamma-2-herpesvirus exhibiting homology with Herpesvirus saimiri and Epstein–Barr virus. This virus was named Kaposi’s sarcoma-associated herpesvirus (KSHV) and provisionally designated human herpesvirus-8 (HHV-8).

Results: KSHV is detectable in more than 90% of classical-Mediterranean, iatrogenic, endemic-African, and AIDS-epidemic KS lesions. In situ PCR studies have demonstrated KSHV in the spindle cells and endothelial cells of KS lesions. KSHV appears to be a transmissible B-lymphotropic herpesvirus. It is detectable in circulating B cells in some HIV-infected patients, and this finding appears to predict the future development of KS among these individuals. KSHV has been identified in a rare and distinct subset of malignant lymphoma referred to as body cavity-based lymphoma but not in other lymphoid neoplasms. KSHV is absent from most other HIV- and non-HIV-associated lymphadenopathies.

Conclusions: Further studies should lead to a better understanding of the role of KSHV in the pathogenesis of these disorders and may eventually show that KSHV represents the long sought-after etiologic agent of Kaposi’s sarcoma.

Key words: body cavity-based lymphoma, human herpesvirus-8, Kaposi’s sarcoma, Kaposi’s sarcoma-associated herpesvirus, multicentric Castleman’s disease

Kaposi’s sarcoma

Kaposi’s sarcoma (KS) has been largely considered an enigmatic clinicopathologic entity since it was discovered by Moritz Kaposi in 1872 [1]. Four clinical-epidemiologic forms of the disease have been described: classical-Mediterranean KS, which occurs very rarely in elderly individuals, predominantly men, of Mediterranean and Eastern European descent, in whom it usually exhibits indolent clinical behavior; endemic-African KS, which occurs commonly in HIV-negative individuals in equatorial Africa, in whom it often exhibits aggressive clinical behavior; iatrogenic KS, which occurs relatively frequently in solid organ transplant recipients, in whom it often regresses following reduction of immunosuppressive therapy; and AIDS-epidemic KS [2], which has emerged as the most common neoplasm in individuals who have AIDS [2] and is a defining condition for that disease [3].

Kaposi’s sarcoma is composed of a variable mixture of ectatic, irregularly shaped, round capillary and slit-like endothelial-lined vascular spaces and spindle-shaped cells accompanied by a variable inflammatory mononuclear cell infiltrate. Red blood cells and hemosiderin pigment are usually present, often extravasated between the spindle cells. The earliest patch and plaque stage lesions sometimes may be difficult to distinguish from granulation tissue. The spindle cells increasingly become the predominant cell population, forming fascicles that compress the vascular slits, and the lesions become progressively nodular [4]. The histopathologic features of the patch, plaque, and nodular lesions of KS are similar among the different clinical-epidemiologic categories of the disease [4]. The histogenesis of the spindle cell component, believed to be the ‘tumor cell’ of KS, has remained controversial, although most investigators favor an endothelial cell origin [5].

A considerable body of clinical observations and epidemiologic data suggests that KS may have an infectious etiology and be transmitted sexually [6, 7]. For example, among AIDS patients, KS occurs almost exclusively among homosexual men, in whom it may be associated with specific sexual practices [6, 7]. In contrast, KS is very uncommon among adult AIDS patients infected through heterosexual or parenteral HIV transmission or among pediatric AIDS patients infected through vertical HIV transmission [5]. Numerous organisms, including cytomegalovirus, hepatitis B virus, human herpesvirus-6, human immunodeficiency virus (HIV), mycoplasma pneumoniae, and human papillomavirus, have been suggested as the etiologic agent of KS [5]. However, a strong etiologic association between any of these infectious organisms and KS has never been demonstrated [5]. Consequently, none of them have been accepted as a causative agent in the pathogenesis of KS.
**Discovery of Kaposi's sarcoma-associated herpesvirus**

Chang, Moore, Cesarman, Knowles, and coworkers utilized representational difference analysis [8] to identify two novel nonhuman DNA sequences, designated KS330Bam and KS631Bam, in KS tissue obtained from a homosexual man who had died from AIDS [9]. They showed that these DNA sequences exhibit partial homology with genes encoding tegument and capsid proteins of Herpesvirus saimiri (HVS) and Epstein–Barr virus (EBV) [9], two herpesviruses belonging to the Gammaherpesvirinae subfamily [10]. The degree of homology suggested that these sequences belonged to a novel, previously unidentified member of the same herpesvirus family. For this reason, and because of its association with KS, this agent was initially named descriptively as Kaposi's sarcoma–associated herpesvirus (KSHV) [9]. It has been provisionally designated human herpesvirus-8 (HHV-8) [11].

**Characterization of KSHV**

Evidence supporting the notion that these DNA sequences are part of a new human herpesvirus has accumulated rapidly. Two lymphoma cell lines derived from AIDS-related body cavity-based lymphomas (discussed below) that retain these sequences were established in our laboratory. These cell lines, designated BC-1 and BC-2, have been used extensively to characterize this viral agent [12]. We discovered that these sequences represent a portion of a much larger DNA molecule that is located in the nucleus of the infected cells. Fluorescence in situ hybridization studies of metaphase spreads prepared from both cell lines demonstrate these sequences in episcopal structures [12]. Their presence as large nuclear episomes in these cells is consistent with a latent herpesvirus genome and, in conjunction with the homology data, strongly suggested that this agent belonged to the herpesvirus family.

Sequence analysis of separate cloned fragments obtained from genomic DNA libraries by Moore et al. [13] and Cesarman et al. [14] further demonstrated that these sequences are part of a large herpesvirus genome. Moore and colleagues [13] used one of the originally described fragments, KS330Bam, to clone a 20.7 kb DNA fragment from a KS lesion. This fragment was entirely sequenced, revealing 17 partial or complete open reading frames (ORF), 16 of which have sequence and positional homology to known gamma-2 herpesvirus genes, including major capsid protein and thymidine kinase genes [13]. Phylogenetic analysis demonstrated that KSHV belongs to the gamma-2 sublineage (genus Rhadinovirus) of the Gammaherpesvirinae subfamily, making it the first member of this genus known to infect humans [13]. Cesarman et al. [14] used the second originally described fragment, KS631Bam, to clone a 13 kb DNA fragment from an AIDS-related body cavity-based lymphoma. Sequence analysis of this fragment revealed four ORFs colinear with similar genes in HVS and present in the same transcriptional orientation. Three of these four ORFs have homologies to known viral and/or cellular genes. These include homologies to (1) EBV and HVS membrane antigens p140 and p160, respectively; (2) HVS and cellular cyclines, predominantly the cyclin D family; and (3) HVS and cellular G-protein coupled receptors [14]. These findings demonstrate that KSHV is colinear with HVS, another gamma-2 herpesvirus, which appears to represent its closest relative.

Subsequently, Mesri et al. [15] demonstrated that KSHV is encapsidated and distinct from EBV and that the KSHV-containing BC-1 lymphoma cell line produces infective viral particles capable of specifically transmitting KSHV to umbilical cord blood CD19-positive B cells but not to CD19-negative cells. This transmission requires a biologically active and replicating herpesvirus, i.e., KSHV, since it is blocked by ultraviolet irradiation, which is known to inactivate herpesviral DNA replication and transcription, and also by fosarnet, a viral DNA polymerase inhibitor. These findings strongly suggest that KSHV is a transmissible B-lymphotropic herpesvirus.

Recently, we established a KSHV-containing, EBV-negative lymphoma cell line, designated BC-3, from a body cavity-based lymphoma arising in an HIV-negative patient [16]. Pulsed field gel electrophoresis of intact cells and viral pellets demonstrate KSHV genomes of 170 kb molecular weight, consistent with herpesviral genomes. Sonication and DNAse treatment studies provide evidence for the encapsidation of these 170 kb viral genomes [16]. We further showed that the BC-3 cells exhibiting viral cytopathic effect contain typical 100 to 115 nm intranuclear herpesvirus nucleocapsids and complete cytoplasmic viral particles, i.e., KSHV [17]. Renne and colleagues [18] demonstrated that the in vitro production of large numbers of KSHV virions can be activated in KSHV-positive, EBV-negative body cavity-based lymphoma cell lines by phosphor esters. These cell lines represent an invaluable source of KSHV for the evaluation of its pathogenic potential as well as characterization of its mechanistic role in the development of KS and malignant lymphoma.

**Association of KSHV with Kaposi’s sarcoma**

To determine the specificity of the two DNA fragments for AIDS-KS, Chang et al. hybridized them by Southern blotting to DNAs extracted from a variety of AIDS and non-AIDS tissues. They found that 78% of AIDS-KS lesions hybridized with variable intensity to KS330Bam and/or KS631Bam. They subsequently designed primers from KS330Bam that amplify a 233 bp fragment (KS330233) and found that 93% of the same AIDS-KS tissues were positive by polymerase chain reaction (PCR) amplification for KS330233, clearly demonstrating that KS330Bam is present in some KS lesions at levels below the threshold for detection by Southern blotting. They also identified the PCR-amplified 233 bp fragment in 3 of 12 AIDS lymph nodes and in 3 of 27 AIDS lymphomas.
but not in any of 29 non-AIDS lymphomas, 7 non-AIDS benign lymph nodes, 5 vascular tumors, 13 biopsy specimens containing AIDS-associated opportunistic infections, or 49 consecutive surgical biopsies [9]. In summary, they showed that these novel sequences are highly associated with AIDS-KS.

Since then, many investigators have confirmed our original observations and identified this PCR-amplified 233 bp fragment in virtually all KS lesions belonging to all four forms of the disease: classical-Mediterranean, endemic-African, iatrogenic, and AIDS-epidemic [19-23]. Moreover, these numerous samples have exhibited a high degree of homology with the published sequence, indicating that the DNA sequence of this virus is highly conserved and that similar viral strains are present in KS lesions worldwide.

These molecular studies cannot demonstrate the cell population within the KS lesions that actually harbors the virus, however. This leaves open the possibility that KSHV resides in an inflammatory cell population associated with KS lesions and not in the KS cells themselves. However, Boshoff et al. [24] have demonstrated by in situ PCR that KSHV is present in the nuclei of the majority of the spindle cells and the flat vascular lining cells in AIDS and non-AIDS cutaneous nodular KS lesions. Thus, KSHV appears to be present within the KS cells generally considered to represent the ‘tumor cells’ of KS and not in innocent bystander inflammatory cells.

**Association of KSHV with malignant lymphoma**

Non-Hodgkin’s lymphoma (NHL) is the second most common neoplasm occurring among persons who have AIDS [25]. The majority of AIDS-related NHLs are diffuse aggressive B-cell lymphomas exhibiting Burkitt, large-cell, or immunoblastic morphology [25]. In addition, Knowles et al. [26] and later other investigators [27, 28] have described an unusual and uncommonly occurring subset of AIDS-related NHLs that grow exclusively or mainly in the pleural, pericardial, or abdominal cavities as lymphomatous effusions, usually in the absence of a tumor mass. These body cavity-based lymphomas appear to exhibit other distinctive clinical as well as biological features, which include immunoblastic morphology, an indeterminate phenotype, clonal immunoglobulin gene rearrangements indicating a B-cell lineage derivation, and EBV, in the absence of c-myc gene rearrangements [29].

In their initial studies, Chang et al. [9] identified KSHV in three malignant lymphomas, prompting us to investigate a large clinically and pathologically diverse panel of cases of AIDS and non-AIDS-related lymphoid neoplasia for KSHV by Southern blotting, PCR, or both. We found that all eight AIDS-related body cavity-based lymphomas, but none of the remaining 185 cases of AIDS and non-AIDS-related NHL, Hodgkin’s disease, and lymphoid leukemia contained KSHV [30]. We subsequently demonstrated that body cavity-based lymphomas rarely occur in HIV-negative individuals also, and these cases similarly contain KSHV [31]. We also failed to identify KSHV in the pyothorax-associated lymphomas (PALs) [32], an uncommon subset of malignant lymphomas, most often reported from Japan, that are associated with long-standing pyothorax resulting from artificial pneumothorax for the treatment of pulmonary tuberculosis [33]. The PALs are body cavity-based but develop as a tumor mass in or near the pleura rather than as a lymphomatous effusion. They similarly are high-grade B-cell lymphomas, exhibit immunoblastic morphology, and contain EBV [33]. These findings strongly suggest a specific link between KSHV and the subset of body cavity-based lymphomas associated with an effusion. These observations have since been confirmed by other investigators [34, 35]. KSHV apparently has been rarely detected in other lymphomas [36, 37], but the significance of these anecdotal reports remains to be determined.

The consistent presence of KSHV in this uncommon and unusual subset of NHLs that grow mainly in the body cavities as lymphomatous effusions without an identifiable tumor mass strongly suggests that they represent a distinct entity. We tested that hypothesis by investigating 19 malignant lymphomatous effusions occurring in the absence of a contiguous tumor mass for their clinical, morphologic, immunophenotypic, viral, and molecular characteristics [38]. We detected KSHV in 15 of the 19 lymphomas. The four KSHV-negative lymphomatous effusions exhibited Burkitt or Burkitt-like morphology and c-myc gene rearrangements and thus appeared to be Burkitt-type lymphomas occurring in the body cavities. The 15 KSHV-positive lymphomatous effusions exhibited a distinctive morphology bridging large-cell immunoblastic lymphoma and anaplastic large-cell lymphoma, and all 12 cases studied lacked c-myc gene rearrangements. In addition, these lymphomas occurred in men (15/15), frequently but not exclusively in association with HIV infection (13/15), in whom homosexuality was a risk factor (13/15), presented initially as a lymphomatous effusion in the absence of a contiguous tumor mass (14/15), remained localized to the body cavity of origin (13/15), expressed CD45 (15/15) and one or more activation-associated antigens (9/10) in the frequent absence of B-cell-associated antigens (11/15), exhibited clonal immunoglobulin gene rearrangements (13/15), contained EBV (14/15) and lacked bcl-2, bcl-6, ras and p53 gene alterations (13/15) [38]. These findings suggest that KSHV infection and c-myc gene inactivation may be mutually exclusive events and potentially represent alternative molecular mechanisms in the development of malignant lymphomas presenting as lymphomatous effusions. Based on these findings, we have proposed replacing the term ‘body cavity-based lymphoma’ with the term ‘primary effusion lymphoma’ since the latter term describes these lymphomas more accurately and avoids confusion with other lymphomas that occur in the body cavities. These findings further suggest that the KSHV-containing primary effusion lymphomas represent a distinct clinicopathologic and biologic entity.

Association of KSHV with other diseases

We and most other investigators have failed to identify KSHV in vascular and lymphatic endothelial-cell proliferations other than KS, including reparative granulation tissue, hemangiomas, lymphangiomas, and angiosarcomas [9, 20, 24, 39, 40]. Therefore, KSHV appears to be specifically associated with the endothelial and spindle cells comprising KS lesions, rather than merely exhibiting a tropism for endothelium. Jin et al. [40] have even recommended that the PCR detection of KSHV serve as a reliable diagnostic marker for distinguishing KS from other vascular lesions.

We and others have identified KSHV in the majority of cases of AIDS- and non-AIDS-associated multicentric Castleman's disease (MCD), whether accompanied by KS or not [41, 42], as well as in their peripheral blood mononuclear cells [42]. Multicentric Castleman's disease is an atypical lymphoproliferative disorder believed to be an immune dysregulation [43]. It occurs in older individuals, predominantly men, in association with a senescent immune system. The patients present with multiple lymphadenopathies and a variety of constitutional and other symptoms. They develop multisystem disease, including autoimmune phenomena, cytopenias, skin rashes, and intercurrent infections [43]. Also, they frequently develop malignancies, most commonly KS and NHL [43]. Kaposi's sarcoma develops in a particularly high proportion of HIV-positive patients who have MCD [42]. The identification of KSHV in AIDS and non-AIDS-associated MCD supports an even closer relationship between MCD and KS than previously hypothesized and suggests a role for KSHV in the pathogenesis of MCD as well.

Luppi et al. [35] identified KSHV in 3 of 15 cases of non-HIV-related angioimmunoblastic lymphadenopathy and in 4 of 5 cases of a peculiar form of non-HIV-related lymphadenopathy characterized by florid follicular hyperplasia with increased vascularity that resembles MCD and certain stages of HIV-related lymphadenopathy. KSHV has been identified only rarely in other non-AIDS-related benign lymphoid proliferations [35, 41, 42, 44] and thus does not appear to be widely distributed among the non-neoplastic lymphoproliferative disorders occurring in the general population.

Last, Rady et al. [45] reported the detection of KSHV in a variety of benign and malignant skin lesions in four HIV-negative organ transplant recipients receiving immunosuppressive therapy. The population from which these patients were selected is not evident from the report, and control patient populations were not studied. Moreover, these findings have been challenged by other investigators who have failed to identify KSHV in such cutaneous proliferations [46].

In summary, KSHV appears to be preferentially and highly associated with KS, primary effusion lymphomas, and MCD occurring in both HIV-infected and non-HIV-infected individuals and to be present only very infrequently in other vascular and lymphoproliferative disorders. However, further studies are clearly needed to delineate the complete clinical spectrum of KSHV infection in both immunocompetent and in immunodeficient individuals.

Pathogenetic significance of KSHV

A fierce debate concerning the pathogenetic significance of KSHV, especially in KS, has already begun [47, 48]. The presence of KSHV in virtually all KS lesions arising in all anatomic sites and belonging to all clinical-epidemiologic forms of the disease occurring worldwide suggests an incontrovertible link between KSHV and KS. However, KSHV is not detectable by PCR in the endo-
Herpesvirus saimiri can transform T cells into malignant lymphomas in New World primates other than those associated with immune deficiency [50]. It is known that EBV immortalizes B cells in vitro, and is associated with various malignant lymphomas, including Burkitt's lymphoma [55]. Many G-protein-coupled receptors are involved in cell growth and differentiation and are involved in malignant transformation [53]. Cytokines are required for cellular division and thus play a key role in cellular proliferation [54]. Some of these homologues are expressed in primary effusion lymphomas and in KS lesions [14], suggesting that KSHV is playing an active biological role in these diseases.

It is also remarkable that the majority of primary effusion lymphomas contain EBV in addition to KSHV [30,38]. The presence of these two viruses in combination appears to be unique to these malignant lymphomas. Perhaps KSHV acts in conjunction with EBV to induce full transformation. It is well known that EBV is capable of immortalizing B cell in vitro but alone may be insufficient for tumor development, as in the case of the complementary role of EBV and an activated c-myc gene in Burkitt's lymphoma [55]. Genetic complementation can occur in vitro with dual viral infections, an example being activation of the EBV replicative cycle by infection with HHV-6 [56]. Alternatively, KSHV may be merely associated with their growth as an effusion. Obviously, as in the case of KS, the precise pathogenic role of KSHV in the development of these primary effusion lymphomas remains to be determined.

The presence of KSHV in virtually all cases of MCD occurring in HIV-positive patients and many cases of MCD occurring in HIV-negative patients [41,42] strongly suggests a role for KSHV in the pathogenesis of this disease as well. However, several points require clarification. For example, given the very high frequency of KS in patients with MCD, sometimes even in the very same lymph nodes involved by MCD, one cannot entirely rule out the possibility that undetected microscopic foci of KS are responsible for some of the cases of apparent KSHV-containing MCD. Alternatively, some of the KSHV-containing MCD lymph nodes may simply represent disseminated KSHV infection in those hosts. Furthermore, in situ hybridization studies have not yet been performed to determine whether KSHV resides in the lymphoid, the vascular, or both components of MCD. Satisfactory resolution of these issues will help clarify the role, if any, of KSHV in the pathogenesis of MCD.

Finally, whether KSHV enjoys a restricted or a ubiquitous distribution has been hotly debated. However, Monini et al. [57] recently reported finding KSHV in more than 90% of ejaculates obtained from HIV-negative immunocompetent men undergoing surgery for varicocele. If confirmed, these findings suggest that KSHV actually infects a large proportion of the healthy adult general population and that the spermatic organs serve...
as the site of that persistent latent infection. This would make KSHV similar to other known human herpesviruses. One obvious implication is that KSHV is transmissible by sexual contact. Another implication is that the mechanisms operational in the pathogenesis of the KSHV-associated diseases are related to viral reactivation rather than primary infection. Obviously, as in the case of the other human herpesviruses, many conditions, including for example, immunosuppression, immune stimulation, and multiple genetic, environmental, and behavioral factors may cooperate in variable combinations in the pathogenesis of the KSHV-associated diseases. Significant additional investigation will be necessary to elucidate these mechanisms.

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