

## KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS-LIKE DNA SEQUENCES IN AIDS-RELATED BODY-CAVITY-BASED LYMPHOMAS

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**Abstract** *Background.* DNA fragments that appeared to belong to an unidentified human herpesvirus were recently found in more than 90 percent of Kaposi's sarcoma lesions associated with the acquired immunodeficiency syndrome (AIDS). These fragments were also found in 6 of 39 tissue samples without Kaposi's sarcoma, including 3 malignant lymphomas, from patients with AIDS, but not in samples from patients without AIDS.

*Methods.* We examined the DNA of 193 lymphomas from 42 patients with AIDS and 151 patients who did not have AIDS. We searched the DNA for sequences of Kaposi's sarcoma-associated herpesvirus (KSHV) by Southern blot hybridization, the polymerase chain reaction (PCR), or both. The PCR products in the positive samples were sequenced and compared with the KSHV sequences in Kaposi's sarcoma tissues from patients with AIDS.

*Results.* KSHV sequences were identified in eight lymphomas in patients infected with the human immuno-

deficiency virus. All eight, and only these eight, were body-cavity-based lymphomas — that is, they were characterized by pleural, pericardial, or peritoneal lymphomatous effusions. All eight lymphomas also contained the Epstein-Barr viral genome. KSHV sequences were not found in the other 185 lymphomas. KSHV sequences were 40 to 80 times more abundant in the body-cavity-based lymphomas than in the Kaposi's sarcoma lesions. A high degree of conservation of KSHV sequences in Kaposi's sarcoma and in the eight lymphomas suggests the presence of the same agent in both lesions.

*Conclusions.* The recently discovered KSHV DNA sequences occur in an unusual subgroup of AIDS-related B-cell lymphomas, but not in any other lymphoid neoplasm studied thus far. Our finding strongly suggests that a novel herpesvirus has a pathogenic role in AIDS-related body-cavity-based lymphomas. (N Engl J Med 1995;332:1186-91.)

IN patients with the acquired immunodeficiency syndrome (AIDS) there is a probability of approximately 40 percent that cancer will develop, especially Kaposi's sarcoma and non-Hodgkin's lymphoma.<sup>1-4</sup> Chang et al.<sup>5</sup> recently found evidence suggesting an infectious cause of Kaposi's sarcoma, an observation consistent with those in previous epidemiologic studies.<sup>1,6-11</sup> Two novel DNA fragments were isolated from the Kaposi's sarcoma lesions of patients with AIDS and identified in more than 90 percent of such lesions, but they were not found in DNA samples from patients without AIDS. These fragments were also identified in 6 of 39 tissues without Kaposi's sarcoma from patients with AIDS, including 3 malignant lymphomas. The base sequence of the DNA fragments and their flanking sequences had partial homology to two herpesviruses, Epstein-Barr virus (EBV) and herpesvirus saimiri, both of which belong to the subfamily of Gammaherpesvirinae.<sup>5</sup> These data suggested the presence in AIDS-associated Kaposi's sarcoma of a previously unidentified human herpesvirus, which we refer to as Kaposi's sarcoma-associated herpesvirus (KSHV).

Most AIDS-related non-Hodgkin's lymphomas are diffuse B-cell lymphomas of the following types: small-noncleaved-cell (Burkitt's and non-Burkitt's) lymphomas (40 percent), large-cell lymphomas (30 percent), and large-cell, immunoblastic plasmacytoid lymphomas (30 percent).<sup>12</sup> Among the less common types found in patients infected with the human immunodeficiency virus (HIV)<sup>13</sup> are the body-cavity-based lymphomas. These may constitute a distinct subgroup be-

cause of their unusual clinical, immunophenotypic, and molecular genetic characteristics.<sup>14-17</sup> They grow exclusively or mainly in the pleural, pericardial, and peritoneal cavities as lymphomatous effusions, usually with no identifiable tumor mass throughout their clinical course. They have indeterminate immunophenotypes but B-cell genotypes with clonal rearrangements of the immunoglobulin genes. Furthermore, unlike many AIDS-related B-cell non-Hodgkin's lymphomas,<sup>18,19</sup> the body-cavity-based lymphomas that were previously analyzed frequently contained EBV and consistently had no rearrangements of the *c-myc* gene.<sup>14,20</sup>

An association between Kaposi's sarcoma and lymphoid cancer has been noted in patients without AIDS,<sup>21,22</sup> and a high risk of malignant lymphoma has been reported in patients with AIDS-related Kaposi's sarcoma.<sup>23</sup> However, the etiologic relation, if any, between these two neoplasms is unknown. Therefore, we investigated a clinicopathologically heterogeneous group of 193 AIDS-related and non-AIDS-related lymphoid neoplasms for KSHV sequences. The neoplasms studied included eight AIDS-related body-cavity-based lymphomas.

## METHODS

### Pathological Samples

A group of 183 well-characterized lymphoid neoplasms (32 of which were from patients with AIDS and 151 of which were not AIDS-related) were selected from among neoplasms obtained in the surgical-pathology laboratory of Columbia-Presbyterian Medical Center. Neoplasms were selected so that most categories of lymphoid neoplasia would be included (Table 1), but the selection was not intended to represent the actual frequency of each category. A preliminary analysis of 56 of these neoplasms (27 AIDS-related and 29 non-AIDS-related non-Hodgkin's lymphomas) had been previously reported.<sup>5</sup> That analysis found KSHV sequences in three AIDS-related lymphomas, all of which were malignant lymphomatous effusions. In the present study, to determine whether KSHV is preferentially associated with AIDS-related body-cavity-based lymphomas, we included 10 additional AIDS-related non-Hodgkin's lymphomas obtained at the Cedars-Sinai Medical Center in Los Angeles, 5 of

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which were based in body cavities. Six of the eight lymphomas of this type analyzed here and found to be KSHV-positive have been described previously. Lymphoma 1 corresponds to Patient 1 described by Chadburn et al.,<sup>20</sup> lymphomas 2 and 3 correspond to Patients 1 and 3 described by Knowles et al.,<sup>14</sup> and lymphomas 4, 7, and 8 were reported by Walts et al.<sup>15</sup> Lymphomas 1, 2, and 3 are the three AIDS-related lymphomas found to be positive for KSHV by Chang et al.<sup>5</sup>

Heparin-treated peripheral blood, samples of effusion and bone marrow aspirate, and tissue-biopsy specimens were collected under sterile conditions during standard diagnostic procedures. Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) density-gradient centrifugation. Representative portions of each tissue specimen were routinely fixed in buffered formalin, B5, or Bouin's solution and embedded in paraffin, and sections stained with hematoxylin and eosin were prepared. The remaining portions were embedded in a cryopreservative solution (OCT, Tissue-Tek, Miles, Elkhart, Ind.) and stored at  $-70^{\circ}\text{C}$ . The diagnosis of each specimen was based on a correlative analysis of the clinical, morphologic, and immunophenotypic characteristics. The specimens were categorized according to the Revised European-American Lymphoma Classification system.<sup>24</sup>

### Immunophenotypic Analysis

The immunophenotypic profiles of all neoplasms included in this study were determined by flow cytometry (FACScan, Becton Dickinson, Mountain View, Calif.); immunoperoxidase staining of frozen tissue sections, cytospin preparations, or paraffin-embedded tissue sections, as previously described<sup>25</sup>; or a combination of these methods. The number and type of antibodies used in each case depended on the antibody panel needed to confirm the diagnosis and the amount of tissue available for study. The antibody panels used to study lymphomas 1, 2, 3, 4, 7, and 8 have been reported previously.<sup>14,15,20</sup> The antibodies used to study lymphomas 5 and 6 included

Dako-LC (leukocyte common antigen, or LCA [CD45]), Leu-14 (CD22), and L26 (CD20) (Dako, Santa Barbara, Calif.); Leu-1 (CD5) (Beckton Dickinson); and AE1/AE3 (cytokeratin) (Boehringer-Mannheim, Indianapolis). Antiserum to the kappa and lambda immunoglobulin light chains (Dako) was used in the analysis.

### DNA Extraction

Genomic DNA was extracted from cryopreserved mononuclear-cell suspensions and tissue blocks by digestion with proteinase K, extraction with phenol-chloroform, and precipitation with ethanol.<sup>26</sup> An alternative salting-out procedure, not requiring organic extraction, was used when a limited amount of tissue was available.<sup>27</sup>

### Southern Blot Hybridization

Five-microgram aliquots of genomic DNA were digested with the appropriate restriction endonucleases according to the manufacturer's instructions (Boehringer-Mannheim), subjected to electrophoresis in 0.8 percent agarose gels, denatured with alkali, neutralized, and transferred to nitrocellulose filters according to the method of Southern.<sup>28</sup> The filters were hybridized as previously described<sup>29</sup> to probes that had been labeled with phosphorus-32 by the random-primer extension method.<sup>30</sup> Autoradiography was performed at  $-70^{\circ}\text{C}$ , once for 16 hours and again for 14 days, to exclude the presence of weak bands.

The presence of KSHV sequences was determined by hybridizing *Bam*HI-digested DNA to <sup>32</sup>P-labeled KS330Bam and KS631Bam probes.<sup>5</sup> To quantitate the sequences, the intensity of the hybridization signal in the DNA samples was compared directly with that of DNA containing known molar amounts of these sequences. The latter was prepared by combining 5  $\mu\text{g}$  of KSHV-negative DNA (from a hyperplastic lymph node) with fivefold serial dilutions of the cloned KS631Bam fragment.

The *c-myc* oncogene was studied by hybridizing DNA digested by *Eco*RI and *Hind*III to a third exon probe (MC413RC).<sup>31</sup> The presence and clonality of EBV infection were determined by hybridizing *Bam*HI-digested DNA to a terminal-repeat probe that detects EBV genomic termini.<sup>32</sup>

### Oligonucleotide Primers and Probes

All the oligonucleotides used for PCR amplification in this study were synthesized by the solid-phase triester method. The sequences of oligonucleotides used in the amplification and hybridization of KS330<sub>233</sub> have been previously reported.<sup>5</sup> The primers and probes for EBV included sets for the *EBNA-2*, *EBNA-3C*, and *EBER* regions and were derived from published sequences.<sup>33</sup>

### Polymerase Chain Reaction and Hybridization

The conditions for the polymerase chain reaction (PCR) and hybridization of KS330<sub>233</sub>Bam were as previously reported.<sup>5</sup> The PCR and hybridizations of EBV were performed as previously described.<sup>34</sup>

### Sequencing of PCR Products

DNA sequencing of the PCR products of KS330<sub>233</sub> was performed on the eight positive lymphomas. The PCR products were sequenced with a *Taq* DyeDeoxy Terminator Cycle Sequencing method with an ABI 373A automated DNA sequencer (Applied Biosystems, Foster City, Calif.). The two strands and two independent PCR products were sequenced to exclude mismatches due to polymerase mistakes.

### Statistical Analysis

Tests of significance were performed by a one-tailed Fisher's exact test with Epi-Info 5.0 (USD, Stone Mountain, Ga.).

## RESULTS

### Southern Blot Analysis of KSHV Sequences

For 191 of the 193 lymphoid neoplasms, DNA samples obtained for diagnostic purposes from 40 HIV-positive and 151 HIV-negative patients were studied by Southern blot hybridization to detect KSHV sequences. Two neoplasms were not examined by Southern blot hybridization because of insufficient DNA. The pathological diag-

Table 1. Results of Screening for KSHV Sequences by Southern Blot Hybridization and PCR.

DIAGNOSIS	NO. OF NEOPLASMS	NO. POSITIVE FOR KSHV		PERCENT POSITIVE
		SOUTHERN BLOT	PCR	
<b>In patients without AIDS</b>				
Chronic lymphocytic leukemia or small lymphocytic lymphoma	8	0	0	0
Monocytoid B-cell lymphoma	2	0	0	0
Follicular lymphoma	12	0	0	0
Diffuse large-cell lymphoma	27	0	0	0
Small-noncleaved-cell lymphoma (Burkitt's and non-Burkitt's)	14	0	0	0
Lymphoblastic lymphoma	4	0	0	0
Anaplastic large-cell lymphoma	13	0	0	0
Plasmacytoma or multiple myeloma	11	0	0	0
Hairy-cell leukemia	3	0	0	0
Acute lymphoblastic leukemia	4	0	0	0
Cutaneous T-cell lymphoma	8	0	0	0
Peripheral T-cell lymphoma	10	0	0	0
Adult T-cell leukemia or lymphoma	5	0	0	0
Hodgkin's disease	7	0	0	0
Post-transplantation lymphoproliferative disorder	23	0	0	0
<b>Subtotal</b>	<b>151</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>In patients with AIDS</b>				
Diffuse large-cell lymphoma	12	0	0	0
Immunoblastic plasmacytoid lymphoma	8	0	0	0
Small-noncleaved-cell lymphoma (Burkitt's and non-Burkitt's)	9	0	0	0
Peripheral T-cell lymphoma	1	0	0	0
Anaplastic large-cell lymphoma	1	0	0	0
Body-cavity-based lymphoma	8	6*	8	100
Hodgkin's disease	3	0	0	0
<b>Subtotal</b>	<b>42</b>	<b>6</b>	<b>8</b>	<b>19</b>
<b>All patients</b>	<b>193</b>	<b>6</b>	<b>8</b>	<b>4</b>

\*Only six of eight neoplasms were examined by Southern blot hybridization because of insufficient DNA in two cases; all six neoplasms tested were positive for KSHV sequences.

noses of the neoplasms are shown in Table 1. Six of the 191 neoplasms tested with probes KS330Bam and KS631Bam were positive for KSHV (Fig. 1, Table 1). All six were body-cavity-based lymphomas from patients with AIDS (chi-square = 160, by Fisher's exact test;  $P < 0.001$ ), which we refer to henceforth as lymphomas 1 through 6. DNA from the six lymphomas had much stronger hybridization signals than DNA from the Kaposi's sarcoma sample used as a positive control (Fig. 1). The approximate copy number of KSHV sequences in each lymphoma was obtained by comparing the hybridization signal for the lymphoma DNA to the signals for DNA containing known molar amounts of KSHV sequences (data not shown). We estimate that there was an average of 1 copy of the KSHV sequence per cell in the Kaposi's sarcoma tissue,<sup>5</sup> as compared with 40 to 80 copies per cell in the malignant lymphomas.

#### PCR Analysis and Sequencing of KSHV in the Body-Cavity-Based Lymphomas

DNA from all 193 lymphoid neoplasms shown in Table 1 was analyzed after PCR with primers KS330<sub>233</sub>F and KS330<sub>233</sub>R, which amplify a 233-base-pair (bp) fragment from the KS330Bam region. Two body-cavity-based lymphomas (lymphoma 7 and lymphoma 8) were analyzed in addition to the 191 neoplasms studied by Southern blotting. All eight body-cavity-based lymphomas were positive for KSHV, but none of the other 185 neoplasms showed an amplified product, even after hybridization with an internal oligonucleotide probe (Table 1, Fig. 2).

To determine similarities in the KSHV sequences from Kaposi's sarcoma tissue and from the body-cavity-based lymphomas, we sequenced the 233-bp PCR band obtained for the eight lymphomas. A very high

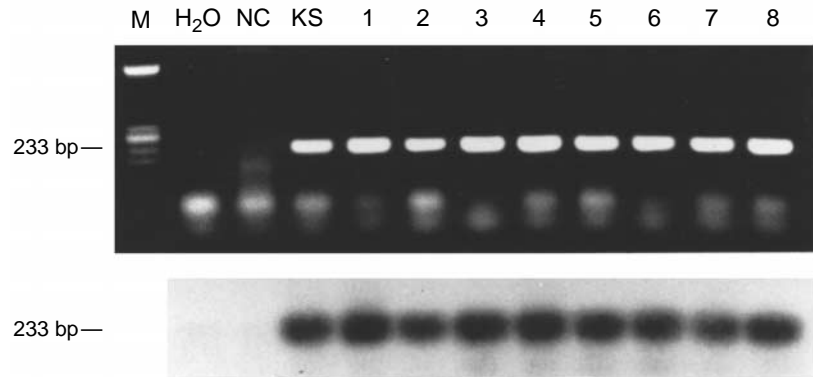


Figure 2. PCR Amplification of DNA from AIDS-Related Body-Cavity-Based Lymphomas, Using the KS330<sub>233</sub> Primers.

The upper panel shows the ethidium bromide-stained agarose gel of the amplification products of DNA from a molecular-weight marker (M; *Hind*III-digested lambda and *Haell*I-digested Phi-X DNA), water (H<sub>2</sub>O), and DNA from a negative control (NC; HL-60 cell line), a positive control (KS), and lymphomas 1 through 8. The lower panel shows specific hybridization of the PCR products to an internal oligonucleotide probe end-labeled with phosphorus-32, after transfer to nitrocellulose filters.

degree of conservation was found. The Kaposi's sarcoma and lymphoma DNA sequences differed only by one to four nucleotides, which resulted in substitutions of one or two amino acids. By contrast, a comparison of these sequences with the analogous region of EBV<sup>35</sup> revealed a difference of 114 bases. Thus, EBV had 51 percent homology to KS330<sub>233</sub> at the DNA level and 35 percent homology at the protein level, whereas homology was greater than 98 percent in the amplified DNA sequences from the Kaposi's sarcoma tissue and the lymphomas. These findings strongly suggest that the sequences obtained by PCR from the body-cavity-based lymphomas correspond to the same putative agent previously identified in Kaposi's sarcoma and differ from sequences in the analogous region of EBV.

#### Clinical, Morphologic, Immunophenotypic, and Molecular Characteristics of Body-Cavity-Based Lymphomas

##### Clinical Features

All eight patients with body-cavity-based lymphomas were homosexual men ranging in age from 30 to 58 years (Table 2). In seven of the eight, the lymphoma presented as a malignant effusion. In Patient 3, a lymphoma in the region of the right submandibular gland was found five months before a lymphomatous pleural effusion appeared. The neoplastic cells in both lymphomas were morphologically and immunophenotypically similar. We were unable to assess the clonal relation of the two tumors or the presence of KSHV sequences in the submandibular-gland lymphoma, because no tissue from the latter was available.

Two patients had a history of Kaposi's sarcoma at the time of the lymphoma diagnosis; none had Kaposi's sarcoma subsequently. No

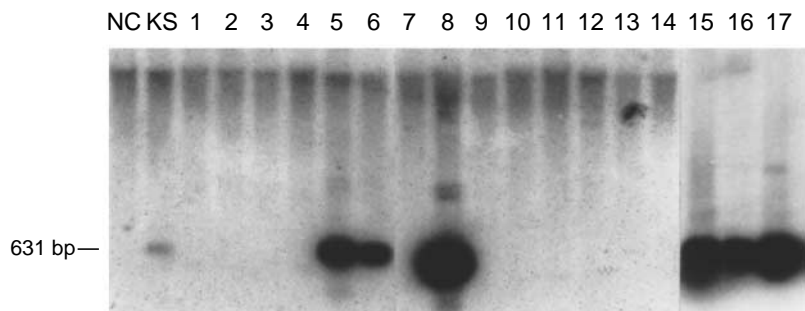


Figure 1. Southern Blot Hybridization to Detect Kaposi's Sarcoma-Associated Viral Sequences.

Lanes 5, 6, 8, 15, 16, and 17 show strong hybridization to probe KS631Bam and correspond to lymphomas 1 through 6, respectively. None of the other lymphomas shown in Table 1 hybridized with this probe. NC denotes negative control (HL-60 cell line), and KS Kaposi's sarcoma DNA (used as a positive control). The same results were obtained with the KS330Bam probe (data not shown).

Table 2. Clinical Characteristics of Eight Homosexual Men with AIDS-Related Body-Cavity-Based Lymphomas.

PATIENT No.	AGE (YR)	SITE OF LYMPHOMA	OTHER TUMORS	OUTCOME	REFERENCE
1	46	Peritoneum	None	Died of lymphoma 12 days after diagnosis	Chadburn et al. <sup>20</sup>
2	31	Pleura	None	Died of HIV-associated meningoencephalitis 7 mo after lymphoma diagnosis	Knowles et al. <sup>14</sup>
3	40	Pleura	Kaposi's sarcoma and submandibular-gland lymphoma	Died 5 mo after initial lymphoma, 10 days after diagnosis of pleural effusion	Knowles et al. <sup>14</sup>
4	35	Peritoneum	None	Died of lymphoma 1 mo after diagnosis	Walts et al. <sup>15</sup>
5	38	Pericardium	None	Died of lymphoma 5 mo after diagnosis	
6	58	Pleura	None	Died of lymphoma 14 mo after diagnosis	
7	30	Pericardium	None	Died by suicide while recovering from pericardiectomy	Walts et al. <sup>15</sup>
8	32	Pleura	Kaposi's sarcoma	Died of disseminated Kaposi's sarcoma 4 mo after lymphoma diagnosis	Walts et al. <sup>15</sup>

Kaposi's sarcoma tissue was available from these patients with which to assess the presence of KSHV sequences. The clinical outcome of all eight patients was poor, with a median survival of three months after the drainage of the malignant effusions.

#### Cytomorphologic Features

The neoplastic cells were round or ovoid to polygonal and contained abundant cytoplasm, usually amphophilic to basophilic, and nuclei that ranged from large, round, and regular to highly irregular and pleomorphic, with one or more large prominent nucleoli. Many cells had plasmacytoid or immunoblastic features. Some binucleated or multinucleated cells resembled Reed-Sternberg cells. Mitotic figures were numerous.

#### Immunophenotypes

All eight body-cavity-based lymphomas expressed CD45, as would be consistent with a hemolymphoid origin. Lymphoma 6 expressed CD20 and CD22, but in all the other lymphomas there was no expression of antigens restricted to the B-cell lineage, and no lymphoma expressed surface or cytoplasmic immunoglobulin. In addition, all eight lymphomas lacked antigens restricted to the T-cell, myeloid, and monocyte lineages. However, there was variable expression of activation-associated antigens HLA-DR, CD23, CD25, CD38, CDw70, and CD71,<sup>14,15,20</sup> and three of five lymphomas studied expressed epithelial membrane antigen, which is often found in immunoblastic lymphomas and anaplastic large-cell lymphomas.<sup>36-38</sup> None expressed cytokeratin. Definitive assignment to the B-cell lineage by immunophenotypic analysis was only possible, therefore, in one of the eight lymphomas.

#### Molecular Genetic Characteristics

All eight lymphomas contained clonal rearrangements of the immunoglobulin gene, establishing their B-cell lineage (Table 3). Lymphomas 1, 2, and 3 were previously shown not to have rearrangements of the *c-myc* gene,<sup>14,20</sup> and Southern blot hybridization of lymphomas 4 and 5 showed germ-line configurations of *c-myc* (data not shown). Lymphomas 6, 7, and 8 were not analyzed because of insufficient DNA. Thus, the *c-myc* oncogene was not rearranged in any of the five AIDS-related lymphomas analyzed.

The presence and clonality of EBV were determined

in six of the eight lymphomas by Southern blot hybridization with a terminal-repeat probe.<sup>32</sup> The detection of a single band in all six (Fig. 3) indicates that EBV infection preceded the clonal expansion of the neoplastic cell population and was present in that population, instead of in normal cells present in the effusion. PCR analysis of the eight lymphomas was performed with primers derived from three regions of the EBV genome (*EBNA-2*, *EBNA-3C*, and *EBER*) to exclude the possibility of cross-hybridization with KSHV sequences and to permit the identification of EBV subtypes A and B.<sup>33,34</sup> All eight lymphomas were positive for EBV with the three sets of primers, whereas the Kaposi's sarcoma samples used as controls were negative (data not shown), thereby ruling out the possibility of cross-hybridization with KSHV sequences. EBV types A and B were both identified (Table 3), as was the case in studies of other AIDS-related non-Hodgkin's lymphomas.<sup>39,40</sup>

## DISCUSSION

This report describes novel Kaposi's sarcoma-associated, herpesvirus-like DNA sequences in a distinct subgroup of AIDS-related malignant lymphomas that are based in body cavities. We identified these sequences in all 8 such lymphomas studied but not in any of 34 other AIDS-related lymphoid neoplasms or in 151 lymphoid neoplasms that were not AIDS-related. The consistent

Table 3. Molecular Analysis of Eight AIDS-Related Body-Cavity-Based Lymphomas Positive for KSHV and EBV Viral Sequences.\*

LYMPHOMA No.	EBV TYPE	ANTIGEN-RECEPTOR GENES				<i>c-myc</i> ONCOGENE
		J <sub>H</sub>	J <sub>K</sub>	C <sub>A</sub>	T <sub>B</sub>	
1	B	R	R	G	G	G
2	A	R	R	G	G	G
3	A	R	R	G	G	G
4	A	R	R	ND	G	G
5	B	R	ND	ND	ND	G
6	A	R	ND	ND	ND	ND
7	A	R	G	ND	G	ND
8	A	R	ND	ND	ND	ND

\*J<sub>H</sub> denotes joining-region probe for immunoglobulin heavy-chain gene, J<sub>K</sub> joining-region probe for immunoglobulin kappa light-chain gene, C<sub>A</sub> constant-region probe for immunoglobulin lambda light-chain gene, T<sub>B</sub> T-cell-receptor beta-chain probe, R rearranged, G germ line, and ND not done.

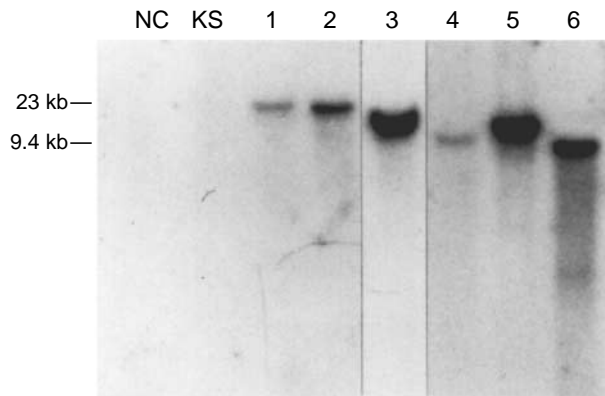


Figure 3. Southern Blot Analysis for the Presence and Clonality of EBV Infection.

The hybridization signals for lymphomas 1 through 6 indicate the presence of EBV. NC denotes negative control (HL-60 cell line), and KS Kaposi's sarcoma DNA (an additional negative control). The detection of a single band with a probe from the terminal-repeat region of EBV demonstrates the presence of this virus in a clonal population.

presence of KSHV sequences in this group of AIDS-related lymphoid cancers and their absence from other categories of lymphoid neoplasia suggest a specific link between body-cavity-based lymphomas and the KSHV sequences. These body-cavity-based lymphomas probably represent a distinct category of AIDS-related lymphomas because of their unusual clinical, morphologic, and immunophenotypic characteristics. The rearrangement of immunoglobulin genes, combined with the expression of cell-surface antigens associated with the late stages of B-cell differentiation, activation, or both in the absence of antigens associated with the early and middle stages of B-cell differentiation, suggests that body-cavity-based lymphomas are the malignant counterpart of a mature stage of B-cell development. These lymphomas are also consistent in containing EBV and in the absence of rearrangement of the *c-myc* gene. All these features, now complemented by the presence of KSHV sequences, support the view that these lymphomas are a distinct biologic entity.

The KSHV sequences analyzed thus far have been homologous with members of the Gammaherpesvirinae subfamily of herpesvirus, which characteristically replicates in lymphoblastoid cells.<sup>41</sup> KSHV is most homologous to EBV<sup>35</sup> and herpesvirus saimiri,<sup>42</sup> with identical sequences for 30 to 50 percent of amino acids. Thus, KSHV is probably a novel member of the subfamily that contains these two viruses. It is well known that EBV immortalizes B cells in vitro and is associated with malignant lymphomas, including endemic Burkitt's lymphoma, AIDS-related non-Hodgkin's lymphomas, lymphoproliferative disorders occurring after transplantation, and Hodgkin's disease.<sup>43</sup> Herpesvirus saimiri, a virus of squirrel monkeys (*Saimiri sciureus*), can be isolated from the peripheral-blood mononuclear cells of healthy animals, but it causes fulminant T-cell lymphomas in New World primates other than its natural hosts.<sup>44</sup> Herpesvirus saimiri can also transform human T lymphocytes so that they grow continuously in

vitro.<sup>45</sup> Thus, the two herpesviruses with the most structural homology to KSHV sequences have the ability to induce latent infection of peripheral-blood lymphocytes of their natural host, immortalize lymphocytes in vitro, and lead to the development of malignant lymphomas. We suggest that KSHV may also be involved in lymphoid transformation.

In addition to KSHV sequences, all eight body-cavity-based lymphomas studied here contained EBV sequences. Since EBV is not found in Kaposi's sarcoma lesions that contain KSHV sequences, the presence of these two viruses in combination appears to be unique to this type of lymphoma. It is well known that EBV can immortalize B cells in vitro, but EBV alone may not be sufficient for tumor development, as is exemplified by the complementation of the activated *c-myc* oncogene and EBV in Burkitt's lymphoma.<sup>46</sup> Thus, it is possible that KSHV acts in conjunction with EBV to induce full transformation. Genetic complementation can occur in vitro with dual viral infections. For example, Flamand et al.<sup>47</sup> reported that infection by human herpesvirus-6 could activate the EBV replicative cycle. Thus, although the specific interactions between EBV and KSHV remain to be assessed, the two viruses may interact with one another to induce neoplastic transformation, certain phenotypic features of body-cavity-based lymphomas, or both.

All the patients with AIDS in this study who had body-cavity-based lymphomas were homosexual men. Since this group is at highest risk for Kaposi's sarcoma, it may also be at highest risk for these lymphomas. However, only two of the eight patients with body-cavity-based lymphomas had Kaposi's sarcoma, an indication that KSHV-associated body-cavity-based lymphomas can occur independently of Kaposi's sarcoma in patients with AIDS.

In conclusion, we have identified Kaposi's sarcoma-associated, herpesvirus-like DNA sequences in all eight body-cavity-based lymphomas we examined. These eight lymphomas have features that distinguish them as a distinct clinicopathological entity. KSHV is also associated with Kaposi's sarcoma, but the neoplastic nature of Kaposi's sarcoma is controversial.<sup>22,48</sup> In contrast, body-cavity-based lymphomas are clearly malignant. The consistent presence of KSHV sequences in these lymphomas suggests that a novel herpesvirus plays a part in their development. Our evidence suggests that KSHV may be a transforming virus in an unusual group of AIDS-related lymphomas.

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