Primary Effusion Lymphoma: A Distinct Clinicopathologic Entity Associated With the Kaposi’s Sarcoma–Associated Herpes Virus

By Roland G. Nador, Ethel Cesnavman, Amy Chadburn, D. Brian Dawson, M. Qasim Ansari, Jonathan Said, and Daniel M. Knowles

We recently discovered the presence of Kaposi’s sarcoma-associated herpes virus (KSHV/HHV-8) in an uncommon and unusual subset of AIDS-related lymphomas that grow mainly in the body cavities as lymphomatous effusions without an identifiable contiguous tumor mass. The consistent presence of KSHV and certain other distinctive features of these body cavity–based lymphomas suggest that they represent a distinct entity. We tested this 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. KSHV was present in 15 of 19 lymphomas. All four KSHV-negative lymphomatous effusions exhibited Burkitt or Burkitt-like morphology and c-myc gene rearrangements and, therefore, appeared to be Burkitt-type lymphomas occurring in the body cavities. In contrast, all 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 which homosexuality was a risk factor (13/13), presented initially as a lymphomatous effusion (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 (13/13), contained Epstein-Barr virus (14/15), and lacked bcl-2, bcl-6, ras and p53 gene alterations (13/15). These findings strongly suggest that the KSHV-positive malignant lymphomatous effusions represent a distinct clinicopathologic and biologic entity and should be distinguished from other malignant lymphomas occurring in the body cavities. Therefore, we recommend that these malignant lymphomas be designated primary effusion lymphomas (PEL), rather than body cavity–based lymphomas, since this term describes them more accurately and avoids their confusion with other malignant lymphomas that occur in the body cavities. We further recommend that these PEL be considered for inclusion as a new entity in the Revised European-American Lymphoma Classification.

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morphologic, immunophenotypic, immunogenotypic, viral, and molecular characteristics.

MATERIALS AND METHODS

Pathologic samples. A group of 19 malignant lymphomatous effusions occurring in the absence of a clinically identifiable contiguous tumor mass, 18 presenting ab initio and one shortly following a distant solid tumor mass, were selected for inclusion in this study. They were chosen from among neoplasms accessioned in the Hematopathology Laboratory of the New York Hospital-Cornell Medical Center and the Departments of Pathology of the University of Texas Southwestern Medical Center and the Cedars-Sinai Medical Center. Some aspects of 11 of these 19 patients have been described previously. Patient no. 1 corresponds to case no. 1 described by Chadburn et al.1 patients no. 2 and 3 correspond to cases no. 1 and 3 described by Knowles et al. patient no. 4 corresponds to case no. 3 reported by Walts et al., patient no. 9 to 13 correspond to the five cases described by Ansari et al., and patient no. 14 was described by Nador et al.2 Patients no. 1 to 6 also correspond to lymphomas 1 to 6 discovered to be KSHV-positive by Cesaran et al.21

Heparin-treated samples of effusions and fresh, unfixed tissue biopsy specimens were collected under sterile conditions during standard diagnostic procedures. Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density-gradient centrifugation. Cytospin preparations of cells isolated from effusion samples were stained with Wright-Giemsa and hematoxylin and eosin. The residual cells were cryopreserved in the presence of fetal calf serum in dimethylsulfoxide at ~170°C in liquid nitrogen. Representative portions of each tissue specimen were routinely fixed in buffered formalin, Bouin’s solution (OCT; Tissue-Tek, Miles Laboratories, Elkart, ID) and stored at -70°C. The diagnosis of each specimen was based on correlation analysis of the clinical, morphologic, and immunophenotypic characteristics.

Immunophenotypic analysis. The immunophenotypic profiles of the samples included in this study were determined by flow cytometry (FACSscan, FACSsort [Becton Dickinson, Mountain View, CA]; Epiics Profile [Coulter, Kennesaw, GA]), immunoperoxidase staining of cytospin preparations, or paraffin-embedded tissue sections, or of cytospin preparations, or paraffin-embedded cell pellets or tissues in cases no. 7, 8, 13, and 18, as previously described.30 Briefly, 3- to 5-mm tissue sections were cut, collected in microfuge tubes, deparaffinized with xylene and alcohol, and subsequently digested with proteinase K (200 mg/mL) at 37°C overnight in proteinase K buffer (50 mmol/L Tris, pH 8.5, 1 mmol/L EDTA, 0.5% Tween 20). The proteinase K was then heat inactivated (95°C for 8 minutes). The samples were centrifuged for 15 minutes at 12,000 rpm, the supernatants transferred to new microfuge tubes, and the DNA concentrations measured. The quality of the DNA used for polymerase chain reaction (PCR) was evaluated by amplifying the p53 gene exon 6 region.

Southern blot hybridization analysis. Five-microgram aliquots of genomic DNA were digested with the appropriate restriction endonucleases according to the manufacturer’s instructions (Boehringer-Mannheim), electrophoresed in 0.8% agarose gels, denatured with alkali, neutralized, and transferred to nitrocellulose filters according to Southern.31 The filters were hybridized as previously described2 to probes that had been 3P-labeled by the random-primer extension method.3 Autoradiography was performed at -70°C for 16 to 48 hours.

The presence of KSHV sequences was determined by hybridizing BamHI-digested DNA to the KS330Bam and KS631Bam probes.34 The presence and the clonality of EBV infection was investigated by hybridization of BamHI-digested DNAs to a probe specific for the EBV genomic termini (5.2-kb BamHI-EcoRI fragment isolated from the fused BamHI-terminal fragment N32). The immunoglobulin heavy-chain (IgH) and kappa and lambda light-chain genes were investigated by hybridization of EcoRI- and HindIII-digested DNAs to an IgH gene joining region (JH) probe.35 BamHI-digested DNAs to a kappa light-chain joining region (Jk) probe,36 EcoRI-digested DNAs to a lambda light-chain constant region (Ca) probe,36 and EBER-digested DNAs to a lambda light-chain constant region (Ca) probe.36 The T-cell receptor beta chain (Tb) gene was investigated by hybridization of EcoRI- and BamHI-digested DNAs to a DNA probe that hybridizes to the constant region of the Tb gene.37 The organization of the c-myc gene was analyzed by hybridization of EcoRl- and HindIII-digested DNAs to the human c-myc probe MC1413RC, representative of the third exon of the c-myc gene.38 The bcl-6 gene truncation was examined by hybridization of BamHI- and Xhol-digested DNAs to the bcl-6-specific Sac I probe.39

Oligonucleotide primers and probes. All of the oligonucleotides used as primers for PCR amplification and as internal oligoprobes for Southern blotting were synthesized by the solid-phase triester method. Pairs of primers derived from published sequences were used to analyze KSHV,7 HIV,40 EBV regions EBNA-2, EBNA-3c, and EBER.41,42 the bcl-2 major breakpoint and minor cluster regions,43,44 the c-myc gene first exon–first intron boundary region,19 p53 gene exons 5 through 8,8,45,46 and H-, K-, and N-ras gene codons 12, 13, and 61.47,48 PCR amplifications and hybridizations. The conditions for the PCRs and hybridizations were as previously described19,40,44,45,50,51

Single-strand conformation polymorphism analysis. Single-strand conformation polymorphism (SSCP) analysis was accomplished according to an adapted version4 of a previously reported method.52 The conditions for SSCP analysis were as previously described.48

Cloning and sequencing of PCR products. PCR products were cloned in the pCR 1000 vector using the TA cloning system (Invitrogen, San Diego, CA) following the manufacturer’s instructions. The sequence analysis was performed by using universal primers with a Taq DyeDeoxy Terminator Cycle Sequencing method with an ABI 373A automated DNA sequencer (Applied Biosystems, Foster City, CA).

RESULTS

KS-associated herpes virus. The presence of KSHV was determined by PCR using primers KS330aF and

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Table 1. Viral Sequences in the 19 Malignant Lymphomatous Effusions

<table>
<thead>
<tr>
<th>Case No.</th>
<th>KSHV</th>
<th>EBV</th>
<th>EBV Type</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Positive</td>
<td>ND</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Positive</td>
<td>ND</td>
<td>B</td>
<td>Negative</td>
</tr>
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<td>9</td>
<td>Positive</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Positive</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Positive</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Positive</td>
<td>ND</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>Positive</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td>Clonal</td>
<td>A</td>
<td>Negative</td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td>Clonal</td>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>18</td>
<td>Negative</td>
<td>Clonal</td>
<td>A</td>
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<tr>
<td>19</td>
<td>Negative</td>
<td>ND</td>
<td>A</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

KS3302:R, which amplify a 233-bp fragment. Thirteen of the 17 lymphomas occurring in HIV-seropositive patients (patients no. 1 to 13) and the lymphomas occurring in both HIV-seronegative patients (patients no. 14 and 15) exhibited a distinct 233-bp band similar to the KS lesion used as a positive control, indicating the presence of KSHV. The remaining four lymphomas (patients no. 16 to 19) lacked an amplification product, even after transfer and hybridization of the gels to a \( ^{32} \text{P} \)-end-labeled internal oligonucleotide probe (Table 1 and Fig 1). To determine similarities in the KSHV sequences we sequenced the 233-bp DNA band obtained from both the malignant lymphoma and a KS lesion occurring in patient no. 13. The sequences of the two 233-bp amplification products were identical.

Clinical features. The principal clinical features of the 19 patients are summarized in Table 2. All 19 patients were men. Seventeen were HIV-seropositive homosexual men ranging in age from 31 to 58 years (median, 40). One also had a history of intravenous drug abuse. The two HIV-seronegative men were 79 and 85 years of age, respectively. A diagnosis of AIDS had been previously established in 11 of the 17 (65%) HIV-seropositive patients on the basis of opportunistic infections (\( n = 6 \)), KS (\( n = 2 \)), or both (\( n = 3 \)). Among the HIV-seropositive patients, the absolute CD4 T-cell counts ranged from 9 to 561 cells/\( \mu \text{L} \) (median, 84). The CD4 T-cell count in HIV-seronegative patient no. 18 was 288 cells/\( \mu \text{L} \) (normal range, 588 to 1,202).

Eighteen of the 19 patients presented initially with a malignant lymphomatous effusion; patient no. 3 presented with a submandibular gland tumor mass 5 months before the development of a malignant pleural effusion. The lymphoma appeared to remain strictly localized to the body cavity of origin in 12 of 14 patients with KSHV-containing and two of four patients with non-KSHV-containing malignant lymphomas. Patient no. 3 had generalized lymph node involvement terminally. Patient no. 8 had local extension into an esophageal lymph node and pulmonary perivascular lymphatic involvement without infiltration into the pulmonary parenchyma. Patients no. 18 and 19 had bone marrow infiltration and, in addition, patient no. 19 had thoracic and abdominal lymph node involvement and developed an abdominal effusion as well. Nine patients were treated with chemotherapy, three patients received only supportive therapy, and the type of therapeutic intervention in the remaining six patients is unknown. Eighteen of the 19 patients died between 12 days and 14 months (median, 5 months) following diagnosis. One patient remains alive with disease at 2 months. There was no difference in survival between the 17...
Table 2. Clinical Characteristics of 19 Patients With Malignant Lymphomatous Effusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>HIV Status</th>
<th>HIV Risk Factors</th>
<th>Absolute CD4 Count (µL)</th>
<th>Location of Effusion</th>
<th>Other Sites of Disease</th>
<th>Therapy</th>
<th>Outcome</th>
<th>References</th>
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<tr>
<td>1</td>
<td>46</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>561</td>
<td>Abdominal</td>
<td>None</td>
<td>C</td>
<td>Died 12 d</td>
<td>Cesarsman et al11; Pt 1</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>65</td>
<td>Pleural</td>
<td>None</td>
<td>C</td>
<td>Died 7 mo</td>
<td>Cesarsman et al11; Pt 2</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>50</td>
<td>Pleural</td>
<td>Submandibular gland, lymph nodes</td>
<td>C</td>
<td>Died 5 mo</td>
<td>Cesarsman et al12; Pt 3</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>60</td>
<td>Abdominal</td>
<td>None</td>
<td>C</td>
<td>Died 1 mo</td>
<td>Cesarsman et al12; Pt 4</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>33</td>
<td>Pericardial</td>
<td>None</td>
<td>U</td>
<td>Died 5 mo</td>
<td>Cesarsman et al12; Pt 5</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>33</td>
<td>Pleural</td>
<td>None</td>
<td>U</td>
<td>Died 14 mo</td>
<td>Cesarsman et al12; Pt 6</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>109</td>
<td>Pericardial</td>
<td>None</td>
<td>U</td>
<td>Died 3 mo</td>
<td>Ansari et al13; Pt 1</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>84</td>
<td>Abdominal</td>
<td>None</td>
<td>U</td>
<td>Died 4 mo</td>
<td>Ansari et al13; Pt 2</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>34</td>
<td>Pleural</td>
<td>Esophageal lymph node, lung</td>
<td>U</td>
<td>Died 2 mo</td>
<td>Ansari et al13; Pt 2</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>25</td>
<td>Pleural</td>
<td>None</td>
<td>C</td>
<td>Died 6 mo</td>
<td>Ansari et al13; Pt 3</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>33</td>
<td>Pleural</td>
<td>None</td>
<td>C</td>
<td>Died 2 mo</td>
<td>Ansari et al13; Pt 4</td>
</tr>
<tr>
<td>12</td>
<td>54</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>134</td>
<td>Pleural</td>
<td>None</td>
<td>C</td>
<td>Alive 2 mo</td>
<td>Ansari et al13; Pt 5</td>
</tr>
<tr>
<td>13</td>
<td>35</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>288</td>
<td>Pleural</td>
<td>None</td>
<td>U</td>
<td>Died 6 mo</td>
<td>Nador et al14</td>
</tr>
<tr>
<td>14</td>
<td>78</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>94</td>
<td>Abdominal</td>
<td>None</td>
<td>U</td>
<td>Died 6 mo</td>
<td>Nador et al14</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>M</td>
<td>+</td>
<td>HIVDA</td>
<td>56</td>
<td>Abdominal</td>
<td>Bone marrow</td>
<td>C</td>
<td>Died 6 mo</td>
<td>Nador et al14</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>60</td>
<td>Abdominal</td>
<td>None</td>
<td>U</td>
<td>Died 7 mo</td>
<td>Nador et al14</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>9</td>
<td>Abdominal</td>
<td>Bone marrow</td>
<td>C</td>
<td>Died 4 mo</td>
<td>Nador et al14</td>
</tr>
<tr>
<td>18</td>
<td>41</td>
<td>M</td>
<td>+</td>
<td>H</td>
<td>200</td>
<td>Pleural, abdominal</td>
<td>Abdominal and thoracic lymph nodes, bone marrow</td>
<td>C</td>
<td>Died 6 mo</td>
<td>Nador et al14</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; H, homosexual; IVDA, intravenous drug abuser; OI, opportunistic infections; C, chemotherapy; U, unknown; Pt, patient.

HIV-seropositive and the two HIV-seronegative patients, or those patients who had KSHV- or non-KSHV-containing malignant lymphomas.

Morphology. Each of the 15 KSHV-containing malignant lymphomatous effusions displayed similar morphologic characteristics (Fig 2). In Wright-Giemsa-stained air-dried cytocentrifuge preparations (Fig 2A and B), the neoplastic cells comprising each of these lymphomas exhibited variable polymorphism and possessed cytomorphic features that appeared to bridge those of large-cell immunoblastic and anaplastic large-cell lymphoma. In each case, the majority of the cells were large, sometimes extremely large, and possessed moderate to abundant amphophilic to deeply basophilic cytoplasm and large round to ovoid nuclei containing one or more large nucleoli. Small round clear cytoplasmic vacuoles were present in occasional cells. A variable number of large pleomorphic cells containing markedly irregular, lobated, and pleomorphic nuclei and cells with immunolastic features were found in every case. These cells often had a jellyfish-like nuclear configuration, with a prominent clear Golgi zone adjacent to the nucleus. Some cells were binucleated and resembled Reed-Sternberg cells. Mitotic figures, sometimes atypical, were abundant.

Hematoxylin and eosin-stained cell block and tissue sections were available for review in four cases. The lymphoma cells appeared somewhat more uniform in size and shape in these histologic sections than in the cytospin preparations (Fig 2C and D). However, the malignant cells similarly were large and displayed variable pleomorphism. They were round or ovoid to polygonal in shape and possessed moderate to abundant lightly eosinophilic cytoplasm and large, round to variably indented nuclei containing one or more prominent eosinophilic nucleoli. Occasional larger pleomorphic cells containing highly irregular to hyperconvoluted nuclei were present. Some of these cells were binucleated and sometimes resembled the Reed-Sternberg cells of Hodgkin's disease. Other cells were multinucleated and had wreath-like nuclei, reminiscent of anaplastic large-cell lymphoma. Numerous mitotic figures, many atypical, were present.

The malignant cells comprising the four malignant lym-
Fig 2. (A and B) Wright-Giemsa–stained air-dried cytocentrifuge preparation of a KSHV-positive primary effusion lymphoma. The cells are considerably larger than normal benign lymphocytes and red blood cells and exhibit cytomorphologic features that appear to bridge large-cell immunoblastic lymphoma and anaplastic large-cell lymphoma. The cells display variable polymorphism and generally possess moderately abundant amphophilic to deeply basophilic cytoplasm. A prominent clear perinuclear Golgi zone is frequently present. Small cytoplasmic vacuoles are occasionally present. The nuclei vary from large and round to highly irregular, multilobated and pleomorphic and often contain ≥1 large prominent nucleoli (original magnification × 630). (C) Hematoxylin and eosin–stained section of a primary effusion lymphoma involving the pulmonary lymphatics (case no. 8) (original magnification × 630). (D) Hematoxylin and eosin–stained pleural fluid cell block section of a KSHV-positive primary effusion lymphoma (original magnification × 400). The neoplastic cells are large, but appear more uniform in size and shape and less polymorphic in these histologic sections than in the cytospin preparations. They contain moderately abundant, lightly eosinophilic cytoplasm, sometimes with a perinuclear hof, and generally large round to ovoid nuclei containing prominent nucleoli.

Fig 3. Wright-Giemsa–stained air-dried cytocentrifuge preparation of a KSHV-negative malignant lymphomatous effusion exhibiting c-myc gene rearrangement. The neoplastic cells are relatively monomorphic and medium-sized, and exhibit cytomorphologic features similar to those of Burkitt-type lymphomas. They possess moderate amounts of basophilic cytoplasm containing numerous small round vacuoles and generally round, regular nuclei, sometimes containing prominent nucleoli (original magnification × 630).

Phenotypic effusions lacking KSHV displayed different cytomorphologic features in Wright-Giemsa–stained cytocentrifuge preparations (Fig 3). These effusions consisted of a relatively monomorphic proliferation of medium-sized cells, although large cells occasionally were present. The cells generally possessed only moderate amounts of basophilic cytoplasm containing variable, but sometimes abundant, numbers of small round vacuoles. These cells generally possessed round, regular nuclei, which often contained one or more prominent nucleoli. In summary, these cytomorphologic features more closely resembled those of Burkitt or Burkitt-like lymphoma.

Immunophenotype. Each of the malignant lymphomatous effusions, including those occurring in HIV-seropositive and HIV-seronegative persons, as well as those containing and those lacking KSHV, exhibited similar immunophenotypic characteristics (Table 3). Eighteen of 19 cases (95%) expressed LCA (CD45). None of the four cases studied expressed TdT, which is associated with the precursor stages of B- and T-cell differentiation.\textsuperscript{55} None of 13, one of 19, and one of 13 cases expressed B-cell–associated antigens CD19, CD20, and CD22, respectively. Three of 13 cases
Type A EBV and those in seven patients contained type B phomatos effusions contained EBV, the exception being patient no. 14, an HIV-seronegative man who had a KSHV-blot hybridization analysis and to permit identification of EBNA-2, and EBER-4'4' to determine the presence of EBV

tion of EcoRI and HindIII-digested DNA to the MC413RC

neoplastic cells comprising any of these 19 lymphomatous

glements in addition to clonal IgH and

rangements, indicating bigenotypism. One case (patient no. 17) displayed the germline configuration of the IgH, k and

light-chain, and Tc genes. However, the clonal origin of this lymphoma was strongly suggested by the presence of a single strong band on Southern blot hybridization to an EBV terminal repeat probe, which indicates the clonal expansion of a single-cell population.‡

c-myc proto-oncogene. All 12 KSHV-containing lymphomatous effusions investigated, 11 occurring in HIV-seropositive and one in an HIV-seronegative patient, exhibited the germline configuration of the c-myc gene on hybridization of EcoRI and HindIII-digested DNA to the MC413RC

c-myc gene probe.38 In contrast, the four lymphomatous effusions lacking KSHV displayed rearrangement of the c-myc gene (Table 4 and Fig 4). Thus, the presence of KSHV and immunoblastic morphology correlated consistently with the germline configuration of the c-myc gene (patients nos. 1 to 15), while the absence of KSHV and Burkitt or Burkitt-like morphology correlated consistently with c-myc gene rearrangement (patients nos. 16 to 19). The presence of KSHV and the occurrence of c-myc gene rearrangement in malignant lymphomas therefore appear to represent mutually exclusive events. All 19 malignant lymphomatous effusions were also investigated for the presence of point mutations or small rearrangements occurring in the first exon–first intron boundary region by SSCP analysis.36 Two cases, one containing KSHV (patient no. 10) and one lacking KSHV (patient no. 18), displayed altered mobility in the SSCP assay, suggesting the presence of mutations in this region of the c-myc gene (Table 4 and Fig 5A). However, sequence analysis demonstrated that the case containing KSHV (no. 10) had a base substitution previously reported in fetal liver DNA,39 and thus most likely represents a polymorphism, rather than a true mutation. The KSHV-negative case (no. 18) had a G-to-T substitution at 45 nucleotides upstream from the first exon–first intron boundary and thus appears to represent a true mutation.

Other proto-oncogene and tumor suppressor gene alterations. The regions spanning 110 to 120 bp across codons 12, 13, and 61 of the H-, K-, and N-ras genes were studied by SSCP analysis58 to determine the presence of ras gene family mutations in these malignant lymphomas. A mutation involving codon 61 (exon 2) of the N-ras gene was identified in case no. 1. This was a CAA-to-CAT transversion leading to a glutamine to histamine amino acid substitution. No other ras gene mutations were identified among these 19 malignant lymphomatous effusions (Table 4 and Fig 5B).

Not a single lymphoma was found to exhibit bcl-2 gene rearrangement based on PCR analysis of the major

Table 3. Immunophenotypic Characteristics of 19 Malignant Lymphomatous Effusions

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCA (CD45)</td>
<td>18/19</td>
</tr>
<tr>
<td>TdT</td>
<td>0/4</td>
</tr>
<tr>
<td>B-cell–associated antigens</td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>0/13</td>
</tr>
<tr>
<td>CD20</td>
<td>1/19</td>
</tr>
<tr>
<td>CD22</td>
<td>1/13</td>
</tr>
<tr>
<td>Ig</td>
<td>3/13</td>
</tr>
<tr>
<td>T-cell–associated antigens</td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>0/10</td>
</tr>
<tr>
<td>CD3</td>
<td>0/16</td>
</tr>
<tr>
<td>CD6</td>
<td>0/13</td>
</tr>
<tr>
<td>CD7</td>
<td>0/8</td>
</tr>
<tr>
<td>Reed-Sternberg cell–associated antigen (CD15)</td>
<td>0/8</td>
</tr>
<tr>
<td>Activation-associated antigens</td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>9/11</td>
</tr>
<tr>
<td>epithelial membrane antigen</td>
<td>4/6</td>
</tr>
<tr>
<td>CD30</td>
<td>7/12</td>
</tr>
<tr>
<td>CD36</td>
<td>9/10</td>
</tr>
<tr>
<td>CD71</td>
<td>8/8</td>
</tr>
</tbody>
</table>

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breakpoint and the minor cluster regions of the \( bcl-2 \) gene.\(^{44,45} \) Similarly, the \( bcl-6 \) gene was found to be in the germline configuration in all 12 cases studied, including those occurring in HIV-seropositive and HIV-seronegative patients, and those containing as well as those lacking KSHV (Table 4).

All 19 malignant lymphomatous effusions were investigated for mutations occurring within exons 5 to 8 of the \( p53 \) gene by SSCP analysis.\(^{46} \) An altered electrophoretic band migration pattern was identified in \( p53 \) gene exon 8 in case no. 9 (Table 4 and Fig 5C). Subsequent cloning and sequence analysis demonstrated that this altered migration pattern corresponded to a C-to-T transition in codon 295, leading to a proline to serine amino acid substitution. No other \( p53 \) gene mutations were identified among these 19 malignant lymphomas.

**DISCUSSION**

We performed a comprehensive analysis of 19 malignant lymphomatous effusions occurring in the absence of a clinically identifiable contiguous tumor mass. This analysis included a critical review of the clinical features of these 19 patients and examination of the morphologic, immunophenotypic, and molecular features of their malignant lymphomatous effusions. Fifteen of the 19 lymphomatous effusions contained KSHV. The four cases lacking KSHV, and only these cases, exhibited \( c-myc \) gene rearrangements, and thus molecularly resembled Burkitt-type lymphomas. These four cases displayed cytomorphic features similar to those of Burkitt or Burkitt-like lymphoma as well. Both AIDS- and non-AIDS-related Burkitt-type lymphomas may secondarily involve the body cavities as lymphomatous effusions\(^{1,3,6} \) and, indeed, two of these four patients had systemic lymphoma. The two remaining patients only possessed body cavity–based disease; however, it is well known that AIDS-related NHLs frequently originate in unusual extranodal sites.\(^{1,3} \) Since these four KSHV-negative lymphomas resemble Burkitt-type lymphomas morphologically and molecularly, we believe that they should be classified as Burkitt-
type lymphomas despite their body cavity involvement. The remaining 15 lymphomatous effusions contained KSHV, lacked c-myc gene rearrangements, and displayed distinctive cytomorphologic features that appear to bridge those of large-cell immunoblastic and anaplastic large-cell lymphoma. They also possess several other features, which, taken together, distinguish them from the pyothorax-associated lymphomas24,25 and all other lymphomas arising and/or occurring in the body cavities, and strongly suggest that they represent a distinct clinical and pathologic entity. We recommend that these malignant lymphomas be designated primary effusion lymphomas (PEL) rather than body cavity-based lymphomas, since this term describes them more accurately and avoids their confusion with the pyothorax-associated lymphomas and other lymphomas that occur in the body cavities.

All 15 patients in this study who had PEL were adult men. Indeed, of more than 30 previously reported patients with an apparent lymphoma of this type4,5,8,21-23 only one woman has been described.8 That patient was 39 years old and HIV-
seropositive, but her AIDS risk factors are unknown. Thirteen of the 15 patients who had PEL were HIV-seropositive. The median age of these patients was 42.5 years, which is slightly older than the median age of 37 to 39 years usually associated with AIDS-related NHL. All 13 HIV-seropositive patients had homosexuality as a risk factor; one patient was also an intravenous drug abuser. HIV-seropositive homosexual men are the AIDS risk group at highest risk for the development of KS, where KSHV sequences are also consistently present. Preexisting KS lesions were identified in five of these 15 patients (33%) with PEL and were frequently present in the previously reported patients with PEL. In one instance, we had the opportunity to compare the KSHV sequences present in both the PEL and KS tissue from the same patient. The sequence of the 233-bp region analyzed was identical in both lesions, suggesting that the same viral isolate was associated with both KS and PEL in this patient. More extensive analyses of such paired samples will be necessary to confirm this finding and its pathogenic significance, especially after highly polymorphic regions of KSHV are identified. However, the absence of KS lesions in 10 patients with PEL clearly demonstrates that malignant lymphoma can be the primary presentation of KSHV infection, in both HIV-seropositive and HIV-seronegative persons.

Although the vast majority of PELs occur in HIV-seropositive individuals, we identified two cases in HIV-seronegative individuals. The only distinguishing feature appeared to be median age at presentation, which was 42.5 years in the HIV-seropositive and 81.5 years in the HIV-seronegative patients. This age distribution is reminiscent of KS, which also occurs at an early age in HIV-seropositive individuals, but usually among the elderly in the HIV-seronegative population. As in the case of sporadic non–AIDS-associated KS, whether some form of immunocompromise is present in these HIV-seronegative patients that predisposes them to the development of this form of malignant lymphoma remains to be determined.

Fourteen of 15 patients with PEL initially presented with a lymphomatous effusion. In contrast, patient no. 3 presented with a solid tumor mass in the submandibular gland region 5 months before the occurrence of a lymphomatous effusion and developed disseminated systemic disease before death. The solid lymphoma mass and the lymphomatous effusion were morphologically and immunophenotypically indistinguishable. However, insufficient material was available to determine whether the preceding solid tumor mass also contained KSHV sequences and/or whether the two tumors were either clonally related or represented clonally distinct primary lymphomas, as has been occasionally described in AIDS patients. This case was classified as PEL and included in this series because it is morphologically, immunophenotypically, and molecularly indistinguishable from the other PELs.

Among the four patients with KSHV-negative lymphomatous effusions, one patient was discovered to have extensive disease outside of the body cavities, including the bone marrow, and a second patient developed bone marrow involvement following the effusion as well. In the two remaining patients, the lymphoma appeared to originate in, and remain strictly localized to, a body cavity. Among the KSHV-containing PELs, in addition to patient no. 3, patient no. 8 developed lymphomatous involvement of a periesophageal lymph node and pulmonary lymphatics. Clearly, not all lymphomas localized to the body cavities truly are PELs and not every PEL remains localized to the body cavities. Therefore, the presence or absence of disease outside of the body cavities cannot be used as the sole criterion to distinguish between KSHV-containing PEL and KSHV-negative Burkitt-type lymphomas occurring as lymphomatous effusions, either primarily or secondarily. Instead, all features, including the cytomorphology of the malignant cells, must be taken into consideration in making this distinction. Determining the presence or absence of KSHV and c-myc gene rearrangement may be necessary in some difficult diagnostic situations.

Evidence has been accumulating to suggest that AIDS-related systemic NHLs may be broadly divisible into two categories, each associated with certain distinct clinical characteristics and exhibiting a distinct constellation of genetic alterations. The Burkitt-type lymphomas appear to occur at an earlier age and in less severely immunodeficient persons, and to be an earlier manifestation of HIV infection and more often a primary manifestation of AIDS. These lymphomas more frequently involve lymph nodes, the bone marrow, and the skeletal musculature. Nearly 100% exhibit c-myc gene rearrangements, two thirds contain p53 gene mutations, one third contain EBV, and virtually none exhibit bcl-6 gene rearrangements. In contrast, immunoblastic lymphomas tend to occur at a later age and in more severely immunodeficient persons, and tend to be a later manifestation of HIV infection and more frequently a secondary manifestation of AIDS. These lymphomas more frequently involve the gastrointestinal tract, CNS, and other extranodal sites. Nearly 100% contain EBV, 25% exhibit c-myc gene rearrangements, 20% display bcl-6 gene rearrangements, and only a few contain p53 gene mutations. AIDS-related large-cell lymphomas more closely resemble the immunoblastic lymphomas than the Burkitt-type lymphomas with respect to these features.

The PELs display a distinct cytomorphologic appearance, described here, resembling immunoblastic and not Burkitt or Burkitt-like lymphoma. The AIDS-related PELs appear to be closely related clinically and molecularly to AIDS-related immunoblastic lymphoma as well. In this series, the AIDS-related PELs occurred in slightly older (median age, 42.5 years) and severely immunodeficient (median CD4 T cells, 84/μL) individuals and as a secondary manifestation of AIDS in nine of 13 patients (69%). Analogous to AIDS-related immunoblastic lymphoma, all 15 AIDS-related PELs contained EBV and all but two lacked ras oncogene or p53 tumor suppressor gene mutations. The latter infrequently observed alterations probably represent secondary genetic events that may be involved in the progression of some PELs, but are not essential for their development. However, the PELs are molecularly distinctive by virtue of their uniform absence of c-myc and bcl-6 gene rearrangements.

Indeed, one striking finding that emerged from this study is that the presence of KSHV and rearrangement of the c-myc gene appear to be mutually exclusive molecular events. This suggests that KSHV and c-myc gene activation may
represent alternative molecular pathways in the development of malignant lymphomatous effusions.

Another distinctive feature of the PELs is their indeterminate (non-B, non-T cell) immunophenotype. The vast majority of NHLs can be assigned to the B- or T-cell lineage based on their expression of various monoclonal antibody-defined lineage-associated antigens. In contrast, the 15 PELs uniformly expressed CD45, consistent with a hematolymphoid derivation, but only one expressed any pan-B-cell antigens associated with the early and middle stages of B-cell differentiation and only three others expressed immunoglobulin. Thus, only four of 15 PELs could be assigned to the B-cell lineage on the basis of their immunophenotypic characteristics. However, all 13 PELs studied exhibited clonal immunoglobulin heavy- and light-chain gene rearrangements, suggesting that they represent an approximately equivalent, mature stage of B-cell development. Moreover, they all expressed a constellation of antigens associated with the late stages of B-cell differentiation and/or activation. These findings suggest that the PELs represent the malignant counterpart of a mature stage of B-cell development, i.e., following antigenic stimulation and lying somewhere between a mature B cell and a plasma cell.

The consistent presence of KSHV in the PELs and its absence in all other categories of lymphoid neoplasia examined thus far strongly suggests that this virus plays an important role in the pathogenesis of this unusual subset of malignant lymphoma. KSHV may represent a transforming virus and hence may play a role in lymphoid transformation in the PELs. The homology data support such a role for KSHV. The two herpes viruses bearing the most structural homology to KSHV, herpes virus saimiri and EBV, possess 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. KSHV has been recently identified in the benign peripheral blood CD19+ B cells of patients with AIDS- and non-AIDS-associated KS. Alternatively, KSHV may be responsible for the growth pattern, i.e., the effusion phenotype, of these PELs, perhaps by causing perturbations in adhesion molecules.

It is also remarkable that virtually every PEL, 14 of 15 cases, contained dual viral sequences, that is, EBV in addition to KSHV. The combined presence of these two viruses also appears to be unique to this unusual subset of malignant lymphoma. Therefore, perhaps KSHV acts in conjunction with EBV to induce full transformation. It is well known that EBV is capable of immortalizing B cells in vitro, but alone may be insufficient for tumor development, as in the case of the complementation of EBV and an activated c-myc gene in Burkitt’s lymphoma. Genetic complementation can occur in vitro with dual viral infections. For example, infection by human herpes virus-6 can activate the EBV replicative cycle. However, the presence of EBV does not appear to be indispensable to the development of PEL, since one PEL occurring in an HIV-seronegative individual lacked EBV. Clearly, the precise pathogenic role for KSHV in the development of PELs remains to be determined; this is currently under active investigation.

In conclusion, our results demonstrate that a strong correlation exists between the presence of KSHV DNA sequences and the development of primary lymphomatous effusions occurring predominantly, but not exclusively, in HIV-infected persons. These lymphomatous effusions possess a unique constellation of features that distinguish them from all other lymphomas occurring in the body cavities, and strongly suggest that they represent a distinctive clinicopathologic and biologic entity. These features are an epidemiologic similarity to KS, including predominant occurrence in men, usually but not always HIV-seropositive, in whom homosexuality is a risk factor, and an older age at presentation in non-HIV- than in HIV-infected patients; origination as a lymphomatous effusion in a body cavity without a contiguous tumor mass; restriction to the body cavity of origin; cells with cytomorphic features bridging those of large-cell immunoblastic and anaplastic large-cell lymphoma; CD45 and activation-associated antigen expression, usually in the absence of B-cell lineage antigens; clonal immunoglobulin gene rearrangements indicating a B-cell genotype; the uniform presence of KSHV and nearly always EBV; and the uniform lack of c-myc gene rearrangements. In view of their distinctive nature, we recommend that they be designated PELs to avoid their confusion with other lymphomas occurring in the body cavities. We further recommend that they be considered by the International Lymphoma Study Group for inclusion as a new entity in the Revised European-American Lymphoma Classification.

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REFERENCES


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Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi’s sarcoma-associated herpes virus

RG Nador, E Cesarman, A Chadburn, DB Dawson, MQ Ansari, J Sald and DM Knowles