Primary Effusion Lymphoma in Women: Report of Two Cases of Kaposi's Sarcoma Herpes Virus-Associated Effusion-Based Lymphoma in Human Immunodeficiency Virus-Negative Women

By Jonathan W. Said, Taizo Tasaka, Seisho Takeuchi, Hiroya Asou, Sven de Vos, Ethel Cesarman, Daniel M. Knowles, and H. Phillip Koeffler

Recent molecular evidence suggests an association with a new herpes virus, Kaposi's sarcoma-associated herpes virus (KSHV/HHV-8), and primary effusion lymphomas (PEL). PELs have a characteristic morphology, phenotype, and clinical presentation with malignant effusions in the absence of a contiguous solid tumor mass. Most cases of PEL have occurred in human immunodeficiency virus (HIV)-positive male patients who are coinfected with Epstein-Barr virus (EBV). This report describes two cases of PEL in HIV- and EBV-negative women. In one patient, a pleural cavity was preceded by classic Kaposi's Sarcoma (KS) of the lower extremities. In the second patient, PEL developed in an artificial cavity related to the capsule of a breast implant. Both cases had the characteristic morphologic appearance of high-grade anaplastic/B-cell immunoblastic lymphomas, with loss of B-cell differentiation antigens, clonal immunoglobulin heavy chain gene rearrangements, and expression of activation antigen CD30. Both cases were negative for EBV, herpes virus simplex, and cytomegalovirus (CMV). DNA extracted from both lymphomas and skin KS specimen showed KSHV sequences by molecular analysis. This report expands the spectrum of KSHV-associated disease to include PEL in HIV-negative women.

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MATERIALS AND METHODS

Methods. Specimens were obtained from surgical biopsy specimens or fluid aspirations and were studied in Giemsa-stained cytospin preparations and direct smears, as well as formalin or B5-fixed paraffin-embedded cell blocks. Diagnosis of each specimen was based on correlating analysis of the clinical, morphological, and immunophenotypic characteristics.

DNA extraction. In case no. 1, genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks using proteinase K digestion, extraction with phenol/chloroform, and precipitation with ethanol as previously described. In case no. 2, DNA was extracted from formalin-fixed tissue embedded in paraffin blocks, as well as from fresh cells that were snap-frozen in liquid nitrogen and dry ice.

Detection of KSHV. Detection of KSHV sequences was performed by polymerase chain reaction (PCR) amplification of KSHV sequences and confirmed by Southern blotting of PCR products using methods previously described. Sequences of oligonucleotides used for the amplifications and hybridization were as follows: 5'- primer, 5'-TGATATCGAGACCTCCTAAGC-3'; 3' primer, 5'-CGTGTTTGTAGCATCCAC-3', which yielded a 620-bp fragment. The PCR reaction was performed as follows: preheating at 94°C, 40 cycles of 40 seconds of denaturation at 94°C, 30 seconds of annealing at 52.5°C, 40 minutes extension at 72°C, and 10 minutes postextension at 72°C. The PCR products were separated on 2% agarose gels, transferred to nylon membranes, and hybridized with an oligonucleotide KSHV probe 5'-TGGACAGCTGTGTGTTGATACCCAT-3' end labeled with γ-32P (dATP) using T4 polynucleotide kinase (GIBCO/BRL, Gaithersburg, MD). As negative controls, we used human bone marrow DNA, RAJI lymphocytes, and HL-60 myeloblasts, which showed no hybridizing band. Cases were run in tandem with known KSHV positive and negative samples, which gave appropriate results. Studies were repeated on two separate occasions with identical results.

Ig and T-cell receptor gene rearrangement studies were performed on Southern blots, using JH and Jc probes for the Ig genes and Jβ1 and Jβ2 probes for the T-cell receptor, as previously described.

Immunophenotypic characterization. Antibodies used for immunophenotypic characterization of the neoplastic lymphoid cells were as follows: Ig κ and λ light chains, epithelial membrane antigen (EMA), CD3, CD20, CD30, CD45, and CD79a (DAKO Corp, Carpinteria, CA); T-cell receptor ββ1 (T-Cell Sciences, Cambridge, MA); and herpes simplex virus types 1 and 2, Epstein Barr virus (EBV) latent membrane protein (LMP-1), and EBV in-situ hybridization for EBER1 (DAKO; see Table 1). Antibodies to CD5 were...
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Table 1. Phenotypic Characterization of PEL

<table>
<thead>
<tr>
<th>Source</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
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<tr>
<td>Kappa</td>
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<tr>
<td>Lambda</td>
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<td>CD30</td>
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<td>CD43</td>
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<td>CD56</td>
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<td>CD79a</td>
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<td>EMA</td>
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<td>Herpes simplex</td>
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<tr>
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<td>Enzo</td>
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<td>EBV EBER*</td>
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<tr>
<td>T-cell receptor βF1</td>
<td>T-Cell Sciences</td>
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<td>lgH</td>
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Abbreviation: ND, not done. *In situ hybridization; all others by immunoperoxidase stain. †lg heavy-chain gene rearrangement as determined by Southern blot.

RESULTS

Case reports. Cases have been briefly mentioned without data in a letter submitted to the editor. Case no. 1 was an 85-year-old Russian female immigrant diagnosed in June 1995 with KS of both legs that extended to the thighs (Fig 1). After radiation therapy, she developed gangrene, requiring bilateral above-knee amputations. Three months later, she was readmitted complaining of increasing shortness of breath and a dry cough caused by a large, bilateral pleural effusion. Thoracentesis showed a malignant exudative effusion containing large anaplastic polyploid lymphoma cells with prominent nucleoli (Fig 2). The patient had neither lymphadenopathy nor tumor mass.

Immunophenotypic studies were characteristic of PEL, with staining for CD20, CD45, and CD30, but were negative for other T- and B-cell markers (Ig light chains, CD3, CD5, CD79a, and CD43) and were negative for CD15 and EMA (Table 1). A clonal band indicating Ig gene rearrangements was detected with the JH probe. The patient was treated palliatively and died 4 months after diagnosis.

Case no. 2 is a 46-year-old premenopausal female who has had bilateral silicone implants for 5 years and complained of recent swelling in the right breast. On examination, no lymphadenopathy, hepatosplenomegaly, palpable breast mass, or nipple discharge was found. Magnetic resonance imaging (MRI) showed no abnormality in the breast tissue, and computed tomography (CT) scan of the chest showed no mediastinal, hilar, or intrathoracic adenopathy. CT scan of the abdomen showed a 2- × 5- × 3.3-cm retroperitoneal fluid collection. A mammogram showed a large fluid accumulation around the implant and between the capsule and the implant. No evidence of rupture of the implant was found.

Fluid was aspirated from around the breast implant on two occasions (250 mL and 120 mL, respectively), which showed large anaplastic malignant lymphoma cells with prominent nucleoli and irregular nuclear outlines. Numerous apoptotic tumor cells were also noted (Fig 3). Malignant cells were negative for Ig κ and λ light chains and for CD3, CD20, CD45, CD79a, T-cell receptor βF1, and CD15. Cells were strongly positive for CD30 and showed membrane staining for CD43. Cells were negative for herpes simplex and EBV LMP by immunoperoxidase staining and for CMV and EBV EBER by in situ hybridization. A clonal band was detected in Southern blot with the Ig JH probe. The Igκ light chain probe showed no rearranged bands.

Detection of KSHV. Using PCR primers specific for KSHV, characteristic 620-bp amplification products were present on ethidium bromide-stained agarose gels, which hybridized with 32P-labeled KSHV sequences on Southern blot after transfer to nylon membranes. KSHV sequences were identified in DNA extracted from lymphoma cells from both cases and from skin KS tissue from case no. 1 (Fig 4). Specificity was confirmed by hybridization with an internal oligonucleotide probe as previously described. In case no. 2, identical KSHV sequences were detected in DNA extracted from the paraffin block and from fresh-frozen cells on two separate occasions.

DISCUSSION

Searching for an infectious agent in KS, Chang and Moore used representational sequential analysis to identify DNA sequences that have been attributed to a new human γ-2 herpes virus now known as KSHV or human herpes virus 8 (HHV-8). These DNA fragments have partial homology with genes of two herpes viruses, herpes virus saimiri (HVS) and EBV. HHV-8 is the first γ-2 herpes virus pathogenic for humans, and its closest known relative on the basis of the abdomen showed a 2- × 5- × 3.3-cm retroperitoneal fluid collection. A mammogram showed a large fluid accumulation around the implant and between the capsule and the implant. No evidence of rupture of the implant was found.

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Fig 1. Advanced KS of both legs (case no. 1).
of available sequences is HVS, a lymphotropic squirrel monkey virus. HVS causes lymphoproliferative disorders in new world primates other than its natural host.

PELs that predominantly involve body cavities in the absence of a tumor mass are also associated with KSHV. PEL occur mostly in adult male patients with HIV and EBV infections, and almost all have homosexuality as a risk factor. HIV-related PELs tend to occur in older patients (most in the fourth decade of life), and at a somewhat later stage in the disease than Burkitt-like lymphomas. The patients are usually severely immunosuppressed (T cells <100/µL) and most have prior manifestations of acquired immune deficiency syndrome (AIDS) including opportunistic infections. The risk group for HIV-related PEL is similar to KS, and KS lesions can be identified in approximately one third of patients with PEL. One case of PEL has been reported in an HIV-positive female patient, whose AIDS risk factors are unknown. Rare cases of KSHV-associated PEL have been
Fig 4. Southern blot from DNA extracted from lymphoma cells and KS skin lesion (case no. 1) and lymphoma cells aspirated from case no. 2 show characteristic 620-bp KSHV sequences. Lane 1, PEL related to breast implant (case no. 2); lane 2, negative control (normal human bone marrow); lane 3, KSHV-positive lymphoma cell line KS-l"; lane 4, KS skin lesion (case no. 1); lane 5, PEL from pleural fluid (case no. 1); lane 6 and 7, KSHV-positive cases (positive controls).

reported in HIV-negative men.\textsuperscript{4,10} KSHV has also been associated with classic KS unassociated with HIV\textsuperscript{13} and in benign lymphoid proliferations in multicentric Castleman's disease.\textsuperscript{14} The spectrum of non-KS tumors associated with KSHV has recently been expanded to include a case of angiosarcoma in an HIV-negative female patient from Hungary.\textsuperscript{15} To our knowledge, the cases reported here are the first cases of KSHV/HHV8-related PEL in HIV-negative women, one of whom was of Russian origin, and whose lymphoma was preceded by classic KS. Availability of DNA from both KS and PEL tissues from this patient allowed comparison of the KSHV product, which was similar for both neoplasms.

Case no. 2 was remarkable in that PEL developed in an effusion between a silicone breast implant and its capsule in the absence of a mass lesion.\textsuperscript{16,17} Breast involvement by non-Hodgkin’s lymphomas is rare and usually presents with a tumor mass. Low-grade breast lymphomas may be related to lymphomas of mucosal-associated lymphoid tissue, although primary and secondary low-, intermediate-, and high-grade lymphomas have been reported.\textsuperscript{17} High-grade lymphomas may have immunoblastic or polymorphic appearance,\textsuperscript{16,17} and neoplastic lymphoid proliferations may be associated with coexistent or antecedent lymphocytic mastitis.\textsuperscript{18} A localized lymphocytic immune reaction with features of recruting lymphadenitis has been described after rupture of a silicone breast implant.\textsuperscript{19}

No evidence is available relating lymphomas to breast prostheses, although lymphoma has been reported in silicone granulomas in lymph nodes, which followed draining prosthetic joint replacements,\textsuperscript{20,21} and a single case has been reported of follicular lymphoma that developed in association with capsular contractions and a palpable nodule medial to a mammary implant.\textsuperscript{22} In case no. 2, we cannot determine whether the effusion was neoplastic de novo or homing occurred of neoplastic lymphoid cells to a preexisting effusion.

The absence of mesothelial cells in this location suggests that these cells are not necessary for proliferation of the neoplastic lymphocytes.

KSHV appears to be predominantly an opportunistic infection, occurring mainly in individuals with immune dysfunction and AIDS. Infection with KSHV has also been documented after bone marrow transplantation.\textsuperscript{23} Herpes virus particles have now been shown by electron microscopy in cell lines derived from HIV-positive and HIV-negative patients with PEL.\textsuperscript{12,24} The majority of patients have disease localized to body cavities (pleural, pericardial, or peritoneum), but occasional cases have involved adjacent organs such as the lung, soft tissues, regional nodes, and bone marrow either at presentation or with advanced disease. Cells in solid tumor masses appear morphologically similar to those in malignant effusions. As with other high-grade lymphoma in immunosuppressed individuals, prognosis is poor, and the majority have died within 1 year of diagnosis.

Our cases resemble rare PELS that have occurred in non-immunosuppressed male patients who are HIV-negative.\textsuperscript{4,10} Neoplastic cells have characteristic staining for common leucocyte common antigen (CD45), with absence of most other B- and T-cell-associated antigens including CD20, CD19, and Ig light chains (Table 1). Ig gene rearrangements confirmed the B-cell lineage of the neoplastic cells.

Lymphomas presenting as effusions are unusual, but not all are associated with KSHV.\textsuperscript{25} Pyothorax-associated lymphomas have been described in the pleural cavity after long-standing inflammation in mine workers and after artificial pneumothorax or tuberculous pleuritis.\textsuperscript{26} Although they may resemble PEL in morphology and in association with EBV,\textsuperscript{27} they are negative for KSHV and usually are associated with a tumor mass.\textsuperscript{4} To make the diagnosis of PEL in these two cases, a combination of clinical, morphological, and phenotypic studies were required, and the association with KSHV/HHV8 was confirmed with molecular techniques.
To our knowledge, these are the first cases of KSHV/HHV8 positive PEL in HIV-negative women, one of whom was of eastern European ethnic origin and whose lymphoma was preceded by classic KS. Case no. 2 is unique in that PEL developed in an effusion related to the capsule of a breast implant. Although the prevalence of KSHV in different population groups is still unknown, these cases indicate that KSHV-related PEL is not restricted to HIV-positive men, and both HIV-positive and HIV-negative women are at risk for this disease.

REFERENCES


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