

Body Cavity–Based Malignant Lymphoma Containing Kaposi Sarcoma–Associated Herpesvirus in an HIV-Negative Man with Previous Kaposi Sarcoma

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Background: The role of Kaposi sarcoma–associated herpesvirus in the development of malignant lymphomas in patients negative for the human immunodeficiency virus (HIV) has not been established.

Objective: To examine the possible role of Kaposi sarcoma–associated herpesvirus in a case of body cavity–based malignant lymphoma that occurred in an HIV-negative patient who had previously had Kaposi sarcoma.

Design: Case study.

Setting: Academic medical center.

Patient: A 94-year-old man with lymphomatous ascites.

Measurements: Polymerase chain reaction (PCR) and Southern blot DNA analysis.

Results: The body cavity–based lymphoma cells were positive for Kaposi sarcoma–associated herpesvirus by PCR and were negative for other herpesviruses, including Epstein–Barr virus, cytomegalovirus, and human herpesviruses 6 and 7. Southern blot analysis of lymphoma DNA showed high levels of Kaposi sarcoma–associated herpesvirus (>40 to 80 genomes/cell). Clonal rearrangement of the immunoglobulin JH and JK genes was present, confirming the presence of a clonal B-cell proliferation.

Conclusions: Kaposi sarcoma–associated herpesvirus may be involved in the development of malignant lymphoma after Kaposi sarcoma in HIV-negative patients. This type of lymphoma, in contrast to body cavity–based lymphoma related to the acquired immunodeficiency syndrome, may have an indolent clinical course.

Kaposi sarcoma–associated herpesvirus is a newly identified herpesvirus found in almost all cases of Kaposi sarcoma that are associated with the acquired immunodeficiency syndrome (AIDS). It is also found in classic Kaposi sarcoma, post-transplantation Kaposi sarcoma, and Kaposi sarcoma that occurs in areas endemic for the disease (1–8). Kaposi sarcoma–associated herpesvirus has also been found in a small subset of AIDS-related lymphomas that are positive for the Epstein–Barr virus (EBV); these lymphomas are called “body cavity–based lymphomas” because they predominantly manifest as malignant effusions (9). Recently, a body cavity–based lymphoma containing Kaposi sarcoma–associated herpesvirus was reported in a patient who was negative for the human immunodeficiency virus (HIV) and had no history of Kaposi sarcoma (10).

We describe an elderly, HIV-negative patient with a history of classic Kaposi sarcoma who developed a body cavity–based lymphoma that was shown to contain Kaposi sarcoma–associated herpesvirus. In contrast to what is usually seen with AIDS-related body cavity–based lymphomas, EBV infection was absent. Epidemiologic studies have suggested that Kaposi sarcoma is associated with the development of secondary lymphoproliferative disorders (11, 12). The case we describe supports the premise that Kaposi sarcoma–associated herpesvirus has a role in the oncogenic transformation of EBV-negative lymphoid neoplasms. Further, the development of a malignant lymphoma positive for Kaposi sarcoma–associated herpesvirus in an HIV-negative patient suggests that this herpesvirus has an etiologic role in the development of secondary neoplasms in association with Kaposi sarcoma.

Case Report

A 94-year-old man was admitted to the Mount Sinai Hospital, New York, New York, for management of massive ascites. The patient’s medical history included resection of a stage II adenocarcinoma of the colon in 1981. Kaposi sarcoma of the right foot had been diagnosed in 1988 and treated with surgical excision. The patient denied having any risk factors for HIV infection, and he had not received any transfusions; the results of serum en-

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zyme-linked immunosorbent assay (ELISA) for antibodies to HIV were negative. The Kaposi sarcoma did not recur. In 1989, the patient presented with pericardial tamponade. The results of pericardiocentesis were not diagnostic. A large left pleural effusion developed, and thoracentesis yielded bloody fluid that contained malignant lymphoma cells. Immunophenotypic studies done by flow cytometry showed a monoclonal B-cell phenotype (CD19-positive, CD20-positive, κ light chain). Computed tomography of the chest, abdomen, and pelvis showed no lymphadenopathy or masses, and the effusion was drained.

The patient did well without receiving specific therapy until 1992, when the left pleural effusion recurred and responded to pleural drainage. Ten days before the patient's final hospitalization in 1995, abdominal distention was noted and examination showed massive ascites. Ultrasonography and computed tomography of the abdomen confirmed the presence of massive ascites without intra-abdominal lymphadenopathy or masses. Chest radiography showed a small left pleural effusion. Abdominal paracentesis was done on admission to the hospital and yielded more than 1 L of fluid that contained malignant lymphoma cells (Figure 1). Fever and abdominal tenderness developed, exploratory laparotomy was done, and an ileal perforation was repaired. The patient's postoperative course was characterized by recurrent fever, intra-abdominal abscess, respiratory failure, and seizures. Peripheral blood T-cell subsets showed marked decreases in the ratio of CD4 cells to CD8 cells (0.1) and in the absolute CD4 cell count (60 cells/mm³). The results of serum ELISA for antibodies to HIV and of polymerase chain reaction (PCR) for HIV RNA were negative. The patient died on the 70th day of hospitalization. Autopsy showed scattered foci of malignant lymphoma that involved the pleura, pericardium, and peritoneum. No recurrent Kaposi sarcoma was found.

Methods

Cells from ascitic fluid were pelleted by centrifugation and stored frozen at -80 °C. Genomic DNA from cryopreserved cells was obtained by digestion with proteinase K, extraction with phenol-chloroform, and precipitation with ethanol (13).

Immunophenotypic studies were done on deparaffinized sections of pelleted ascitic fluid and on autopsy tissue by using the avidin-biotin immunoperoxidase technique (14). The antibodies used were obtained from DAKO (Carpinteria, California) (CD45 [LCA], CD20 [L26], epithelial membrane antigen, HLA-DR, CD68 [KP-1], CD30 [KI-1], CD45RO [UCHL1], κ and λ light chains) and

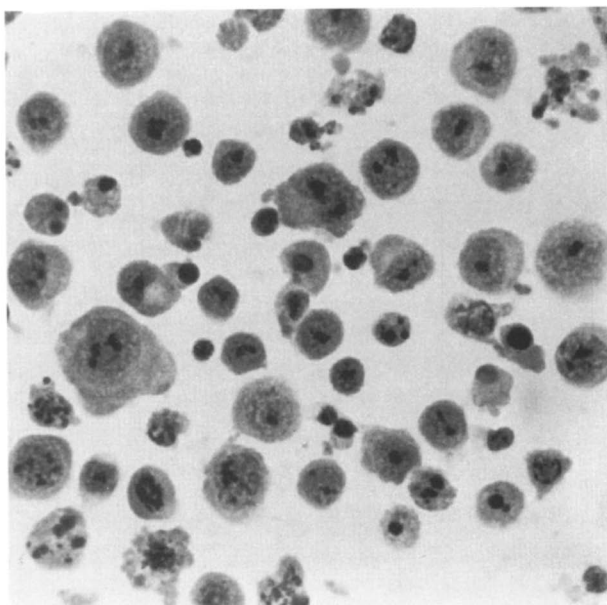


Figure 1. Cytopsin preparation of ascitic fluid cells treated with Papanicolaou stain. Large lymphoma cells with pleomorphic nuclei and prominent nucleoli (original magnification, $\times 400$).

Becton-Dickinson (Mountain View, California) (anti-cytokeratin [CAM 5.2]).

We did PCR amplifications on genomic DNA extracted from ascitic cells. To detect Kaposi sarcoma-associated herpesvirus, we used the primer set for KS330233, as described by Chang and colleagues (1). The primers for EBV targeted the *IR-1* (15) and *EBER* gene loci (16). Primers and PCR conditions for cytomegalovirus and human herpesviruses 6 and 7 are described elsewhere (17, 18).

Kaposi sarcoma-associated herpesvirus analysis was done by Southern blot hybridization using the KS631Bam probe (1) on *Bam*HI digests. Immunoglobulin gene rearrangement analysis was done using JH and JK probes on *Hind*III and *Eco*RI digests. The genomic organization of *c-myc* was investigated using an exon 3 probe (19).

Results

The lymphoma cells in the ascitic fluid cell block and in the autopsy tissue showed a "null cell" immunophenotype with loss of B-cell antigens (CD45 focally positive, CD20-negative, CD45RO-negative, CD30-negative, HLA-DR-negative, CD68-negative, epithelial membrane antigen-positive, cytokeratin-negative, κ and λ immunoglobulin light chain-negative).

The cells from the ascitic fluid were positive for Kaposi sarcoma-associated herpesvirus by PCR. They were negative for several other herpesviruses for which we tested, including EBV, cytomegalovirus, and human herpesviruses 6 and 7.

High numbers of copies of Kaposi sarcoma-

associated herpesvirus DNA sequences were present in the ascitic fluid cells (as determined by Southern blot hybridization) when compared with similar amounts of DNA from the BC-1 cell line, which has approximately 40 to 80 copies of viral genome per cell (Figure 2). The presence of *c-myc* rearrangement was tested using a probe representative of *c-myc* exon 3 that is known, on *Hind*III and *Eco*RI digestion, to detect most *c-myc* rearrangements in AIDS-related non-Hodgkin lymphoma (19). No abnormally migrating band indicative of *c-myc* rearrangement was seen. Despite the lack of surface lineage markers on the ascitic fluid cells, analysis of the JH and JK loci with *Hind*III and *Eco*RI confirmed the presence of a clonal rearrangement showing that the malignant cells were clonal and of B-cell lineage.

Discussion

Kaposi sarcoma-associated herpesvirus DNA sequences were first detected in patients with Kaposi

LANES 1 2 3

631 bp

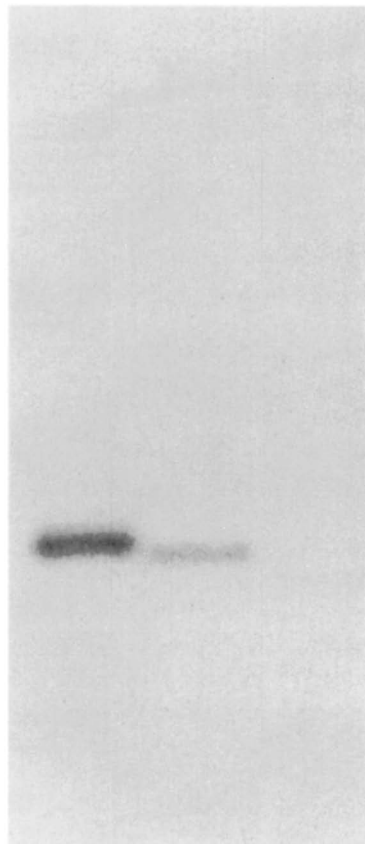


Figure 2. Southern blot of Kaposi sarcoma-associated herpesvirus in malignant ascitic cells. Genomic DNA samples were digested with *Bam*HI and subjected to Southern blot hybridization with the KS631Bam probe. Lane 1 shows ascitic cells from the patient. The BC-1 cell line was used as a positive control (lane 2); P3HR1, a B-cell line positive for Epstein-Barr virus and negative for Kaposi sarcoma-associated herpesvirus, was used as a negative control (lane 3). In the ascitic cells and in the BC-1 cell line, a strong band migrating at 631 base pairs (bp) is shown.

sarcoma and AIDS (1). They have subsequently been identified in cases of Kaposi sarcoma not associated with HIV infection (2–8) and in some lymphoproliferative disorders, including AIDS-related body cavity-based lymphoma (9), multicentric Castleman disease in persons with and without HIV infection (20), and a recent case of body cavity-based lymphoma not associated with HIV infection (10). We describe an HIV-negative patient with a history of classic Kaposi sarcoma who presented with a body cavity-based lymphoma, the cells of which contained many copies of Kaposi sarcoma-associated herpesvirus, as determined by Southern blot hybridization.

Body cavity-based lymphoma is a distinct subtype of non-Hodgkin lymphoma that occurs predominantly in association with HIV infection and has distinctive clinical, immunophenotypic, and molecular genetic features (9). Body cavity-based lymphoma presents predominantly as malignant effusions (“liquid lymphomas”) in the pleural, pericardial, and peritoneal cavities without significant mass or lymphadenopathy. Most body cavity-based lymphomas do not express surface B-cell antigens, but B-cell lineage is indicated by the presence of clonal immunoglobulin gene rearrangement (9). For our patient, B-cell surface antigens were detected by flow cytometry on cells from the pleural effusion in 1989, but they were not detected by immunohistochemical tests on cells from ascitic fluid. B-cell lineage of the ascitic fluid cells was confirmed by the presence of clonal rearrangement of the immunoglobulin heavy- and light-chain genes.

Our patient was HIV-negative on two serum ELISAs and one PCR analysis. He had no identifiable risk factors for HIV infection. The cause of his CD4 lymphopenia has not been established; his lymphocytes were studied only in the period after his bowel perforation, when the confounding factors of sepsis and stress were present. The indolent clinical course of the lymphoma and the prolonged response to drainage of the effusions without other specific therapy also distinguishes our patient’s lymphoma from that typically seen in patients with AIDS (9).

The presence of Kaposi sarcoma-associated herpesvirus in body cavity-based lymphoma suggests a possible pathogenic role for the virus in the development of this neoplasm (9). Kaposi sarcoma-associated herpesvirus shows sequence homology to EBV and herpesvirus samiri, both of which can transform lymphocytes; infection with both of these viruses has also been associated with the development of malignant lymphoma. Kaposi sarcoma-associated herpesvirus in AIDS-related body cavity-based lymphomas has invariably been associated with the presence of EBV (9), which suggests that co-infection

with these two viruses has a pathogenic role. In our patient and in the only previously reported case of body cavity-based lymphoma in an HIV-negative patient (10), EBV was not detected; this suggests that Kaposi sarcoma-associated herpesvirus alone may be lymphomagenic.

Kaposi sarcoma, both classic and AIDS-related, is associated with an increased incidence of secondary neoplasms, particularly such lymphoproliferative disorders as malignant lymphoma and multicentric Castleman disease (11, 12). The detection of Kaposi sarcoma-associated herpesvirus in our patient, who had body cavity-based lymphoma after having Kaposi sarcoma, and in patients with multicentric Castleman disease (20) suggests that Kaposi sarcoma-associated herpesvirus has an etiologic role in the development of Kaposi sarcoma-associated lymphoproliferative disorders.

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References

1. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 1994;266:1865-9.
2. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N Engl J Med*. 1996;332:1181-5.
3. Schalling M, Ekman M, Kaaya EE, Linde A, Biberfeld P. A role for a new herpes virus (KSHV) in different forms of Kaposi's sarcoma. *Nat Med*. 1995;1:707-8.
4. Boshoff C, Whitby D, Hatzioannou T, Fisher C, van der Walt J, Hatzakis A, et al. Kaposi's sarcoma-associated herpesvirus in HIV-negative Kaposi's sarcoma [Letter]. *Lancet*. 1995;345:1043-4.
5. Lebbe C, de Cremoux P, Rybojad M, Costa da Cunha C, Morel P, Calvo F. Kaposi's sarcoma and new herpesvirus [Letter]. *Lancet*. 1995;345:1180.
6. Collandre H, Ferris S, Grau O, Montagnier L, Blanchard A. Kaposi's sarcoma and new herpesvirus [Letter]. *Lancet*. 1995;345:1043.
7. Dupin N, Grandadam M, Calvez V, Gorin I, Aubin JT, Havard S, et al. Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. *Lancet*. 1995;345:761-2.
8. Chang Y, Ziegler J, Wabinga H, Katangole-Mbidde E, Boshoff C, Schulz T, et al. Kaposi's sarcoma-associated herpesvirus and Kaposi's sarcoma in Africa. Uganda Kaposi's Sarcoma Study Group. *Arch Intern Med*. 1996;156:202-4.
9. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med*. 1991;332:1186-91.
10. Nador RG, Cesarman E, Knowles DM, Said JW. Herpes-like DNA sequences in a body-cavity-based lymphoma in an HIV-negative patient [Letter]. *N Engl J Med*. 1995;333:943.
11. Safai B, Mike V, Giraldo G, Beth E, Good RA. Association of Kaposi's sarcoma with second primary malignancies: possible etiopathogenic implications. *Cancer*. 1980;45:1472-9.
12. Biggar RJ, Curtis RE, Cote TR, Rabkin CS, Melbye M. Risk of other cancers following Kaposi's sarcoma: relation to acquired immunodeficiency syndrome. *Am J Epidemiol*. 1994;139:362-8.
13. Sambrook J, Fritsch EF, Maniatis T. *Biomolecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
14. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem*. 1981;29:577-80.
15. Mitchell SM, Fox JD, Tedder RS, Gazzard BG, Lightman S. Vitreous fluid sampling and viral genome detection for the diagnosis of viral retinitis in patients with AIDS. *J Med Virol*. 1994;43:336-40.
16. Lin JC, Lin SC, De BK, Chan WC, Evatt BL, Chan WC. Precision of genotyping of Epstein-Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBV): predominance of type A virus associated with Hodgkin's disease. *Blood*. 1993;81:3372-81.
17. Frenkel N, Rapaport D. PCR: protocols for diagnosis of human and animal virus disease. In: Becker Y, Darai G, eds. *Diagnosis of Human Viruses by Polymerase Chain Reaction Technology*. (Frontiers of Virology; v. 1). New York: Springer-Verlag; 1992:243-52.
18. Rowley AH, Wolinsky SM, Relman DA, Sambol SP, Sullivan J, Terai M, et al. Search for highly conserved viral and bacterial nucleic acid sequences corresponding to an etiologic agent of Kawasaki disease. *Pediatr Res*. 1994;36:567-71.
19. Ballerini P, Gaidano G, Gong JZ, Tassi V, Saglio G, Knowles DM, et al. Multiple genetic lesions in acquired immunodeficiency syndrome-related non-Hodgkin's lymphoma. *Blood*. 1993;81:166-76.
20. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood*. 1995;86:1276-80.

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