

Brief Report

PRIMARY-EFFUSION LYMPHOMA
AND KAPOSI'S SARCOMA IN A
CARDIAC-TRANSPLANT RECIPIENT

DAN JONES, M.D., PH.D., MARY E. BALLESTAS, PH.D.,
KENNETH M. KAYE, M.D., JAMES M. GULIZIA, M.D., PH.D.,
GAYLE L. WINTERS, M.D., JONATHAN FLETCHER, M.D.,
DAVID T. SCADDEN, M.D., AND JON C. ASTER, M.D., PH.D.

HUMAN herpesvirus 8 (HHV-8) is a recently discovered virus that appears to have a pathogenic role in Kaposi's sarcoma, multicentric Castleman's disease, and primary-effusion lymphoma, a distinctive lymphoma that arises within body-cavity effusions.¹⁻⁷ HHV-8 was originally detected in lesions of Kaposi's sarcoma in patients infected with the human immunodeficiency virus (HIV) but was subsequently found in forms of the tumor that occur in HIV-negative patients.^{8,9} Similarly, HHV-8-associated multicentric Castleman's disease occurs predominantly in patients with the acquired immunodeficiency syndrome (AIDS) but also in HIV-negative patients.^{5,10,11} These findings suggest a direct causal role of HHV-8 in the development of Kaposi's sarcoma and multicentric Castleman's disease, with HIV-induced immunosuppression serving as a secondary factor.

Like HIV-infected patients, recipients of solid-organ allografts are at increased risk for a variety of tumors, presumably because of the immunosuppressive regimens used to prevent graft rejection. Kaposi's sarcoma develops in up to 1 percent of organ-transplant recipients and is highly associated with HHV-8 infection.¹²⁻¹⁴ In contrast, HHV-8 infection is unusual in transplant recipients with other tumors, including non-Hodgkin's lymphoma.¹⁵⁻²⁰ HHV-8 infection is also rarely associated with non-Hodgkin's lymphoma in HIV-negative patients, even in southern Italy, where HHV-8 seropositivity is relatively common.^{3,11,21-25} Only a few HHV-8-associated lymphomas have been reported in HIV-negative patients, nearly all of them primary-effusion lymphomas.^{26,27}

From the Department of Pathology (D.J., J.M.G., G.L.W., J.F., J.C.A.) and the Division of Infectious Diseases (M.E.B., K.M.K.), Brigham and Women's Hospital and Harvard Medical School; and the AIDS Research Center, Massachusetts General Hospital and Harvard Medical School (D.T.S.) — all in Boston. Address reprint requests to Dr. Aster at the Department of Pathology, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

©1998, Massachusetts Medical Society.

We describe an unusual case of primary-effusion lymphoma associated with HHV-8 infection in an HIV-negative recipient of a cardiac transplant. The primary-effusion lymphoma was preceded by Kaposi's sarcoma and persistent polyclonal plasmacytic infiltrates involving multiple organs, including the cardiac allograft.

CASE REPORT

The patient was a 59-year-old Haitian man who underwent orthotopic heart transplantation in December 1989 because of end-stage congestive heart disease. Laboratory studies performed before transplantation showed polyclonal hypergammaglobulinemia (serum gamma globulin concentration, 3010 mg per deciliter; normal range, 518 to 1200). The results of an enzyme-linked immunosorbent assay for HIV antibodies and a Western blot assay for HIV type 1 antigens, both performed at another laboratory, were reported to be weakly positive in serum samples obtained at the time of transplantation. However, studies performed at our institution, including enzyme-linked immunosorbent assays for serum HIV antibodies, a polymerase-chain-reaction (PCR) assay for HIV sequences in serum, and viral cultures, were all negative. On the basis of these findings, the patient was considered to be HIV-negative.

The patient had no identifiable risk factors for HIV infection, such as homosexual activity, intravenous drug use, or prior blood transfusion. He had married once and was the father of eight children. He had frequently visited Haiti after his emigration to the United States at the age of 32 years.

Gross and microscopical examination of the explanted heart showed findings consistent with the presence of an idiopathic dilated cardiomyopathy. There were prominent perivascular and interstitial infiltrates of eosinophils and lymphocytes — findings suggestive of drug-induced hypersensitivity myocarditis.

In May 1990, a biopsy confirmed the presence of Kaposi's sarcoma in an inguinal lymph node. At this time, the immunosuppressive regimen consisted of azathioprine (25 to 50 mg per day), cyclosporine (a dose resulting in a blood concentration of approximately 200 µg per deciliter), and prednisone (8 mg per day). Kaposi's sarcoma appeared in the skin in October 1991 and remitted with a reduction in the immunosuppressive regimen (blood cyclosporine concentration, approximately 50 µg per deciliter). The absolute CD4 count in October 1990 was 576 cells per cubic millimeter, with follow-up CD4 counts of 480 cells per cubic millimeter in March 1993 and 510 cells per cubic millimeter in April 1995.

Beginning in August 1991 and continuing through 1997, endomyocardial-biopsy specimens revealed persistent interstitial polyclonal lymphoplasmacytic infiltrates of mild-to-moderate intensity, which were considered atypical of the usual findings in endomyocardial-biopsy specimens obtained after transplantation, such as evidence of rejection, endocardial lymphoid infiltrates, evidence of healing of previous biopsy site, or ischemic injury. Immunohistochemical stains showed the presence of approximately equal numbers of plasma cells expressing kappa and lambda immunoglobulin light chains, a finding consistent with the presence of a polyclonal reactive process. Serum gamma globulin concentrations increased further and were persistently elevated throughout the post-transplantation course (range, 3800 to 4300 mg per deciliter). Serum electrophoresis showed minor biconal kappa M spikes in February 1994 (total IgG, 910 mg per deciliter) and again in October 1997 (total IgG, 790 mg per deciliter), in each instance superimposed on a broad polyclonal IgG peak. Bone marrow biopsy in February 1994 showed only a mild polyclonal plasmacytosis, and the results of urinary protein electrophoresis were unremarkable.

Other conditions that developed in the post-transplantation period included refractory hypertension, chronic renal insufficiency that was presumably due to cyclosporine therapy, and atrial fibrillation.

The patient presented in October 1997 with abdominal pain and dyspnea of several months' duration that was thought to be due to worsening congestive heart failure. The immunosuppressive regimen at this time consisted of cyclosporine (blood concentration, 100 to 200 μg per deciliter) and prednisone (8 mg per day). A chest radiograph showed a prominent left-sided pleural effusion. Thoracentesis revealed a cell-rich effusion (18,550 cells per cubic millimeter) containing a high concentration of lactate dehydrogenase (13,700 IU per liter). The concomitant serum lactate dehydrogenase concentration was also markedly elevated (1526 IU per liter; normal range, 107 to 231). On cytologic examination of the pleural fluid, more than 95 percent of nucleated cells were found to be atypical lymphoid cells with a plasmacytoid appearance. Whole-body computed tomographic scanning confirmed the presence of new bilateral pleural effusions and cardiomegaly that was unchanged from previous examinations, but there was no evidence of tumor masses or lymph-node enlargement. The results of a skeletal survey were normal. The patient declined a bone marrow examination.

On the basis of the cytologic findings and the results of immunophenotypic, genotypic, and ultrastructural studies (described below), a diagnosis of primary-effusion lymphoma was made. Initial treatment consisted of a reduction in the blood cyclosporine concentration to 50 to 100 μg per deciliter, therapeutic thoracentesis, and one cycle of cyclophosphamide, vincristine, and prednisone. Because of rapid reaccumulation of pleural fluid, pleurodesis with bleomycin was performed, followed by a course of cyclophosphamide, doxorubicin, vincristine, and prednisone and two cycles of ifosfamide and etoposide. A partial reduction in the serum lactate dehydrogenase concentration and partial resolution of the effusions were noted, but cytologic examinations of pleural fluid in February and March 1998 showed persistent primary-effusion lymphoma. The patient died of multisystem organ failure and respiratory compromise, with persistent pleural effusions, in April 1998, six months after the diagnosis of lymphoma had been made. An autopsy was declined.

METHODS

Cytologic, Immunophenotypic, and Ultrastructural Studies

Cytologic studies were performed with Wright-Giemsa staining. Immunoperoxidase studies were performed on formalin-fixed, paraffin-embedded cell blocks with the use of antibodies to CD30 (Ber-H2), epithelial membrane antigen, CD45 (leukocyte common antigen), the B-cell markers CD20 (L26) and CD79a, the T-cell markers CD45RO (UCHL1) and CD3 (all from Dako, Carpinteria, Calif.), and Epstein-Barr virus (EBV) latent membrane protein 1. In situ hybridization was performed with a ribonucleotide probe for EBV-encoded transcripts (BioGenex, San Ramon, Calif.), according to the manufacturer's instructions. Analysis of cell-surface markers was performed by flow cytometry on fresh samples of pleural fluid, as previously described, with monoclonal antibodies directed against CD3, CD5, CD10, CD13, CD14, CD19, CD20, CD33, CD38, and CD45 (all from Becton Dickinson, San Jose, Calif.) and CD11b (Immunotech, Marseilles, France).²⁸ For ultrastructural analysis, cells from fresh samples of pleural fluid were fixed first in paraformaldehyde-glutaraldehyde and then in osmium tetroxide, embedded in Epon, sectioned, and stained with uranyl acetate. Structural features were compared with those of primary-effusion-lymphoma-derived cells that have been shown to support the replication of HHV-8.²⁹

For immunofluorescent staining, tumor cells from pleural-effusion fluid were fixed on glass slides in a 1:1 solution of acetone and methanol at -20°C . The cells were incubated with or without immune serum from an HHV-8-infected patient with Kaposi's sarcoma, stained with fluorescein isothiocyanate-conjugated antihuman IgG antibody, and then examined with the use of confocal microscopy. The lymphoma cell lines BC-1³⁰ and BCBL-1,³¹ which are positive for HHV-8, were used as positive

controls, and the EBV-infected cell lines IB4 and B95-8, which are negative for HHV-8, were used as negative controls.

Molecular and Karyotypic Analyses

DNA was purified from pleural-effusion samples by proteinase K digestion and phenol extraction. DNA was isolated from sections prepared from archival formalin-fixed, paraffin-embedded tissue by proteinase K digestion followed by purification on QIAamp silica columns (Qiagen, Chatsworth, Calif.). A 233-bp HHV-8 genomic sequence (KS330Bam) was amplified from 1 μg of genomic DNA by a PCR assay with the use of a previously described pair of primers.³² Control primers from the human BCL2 locus were used to confirm that the template DNA could be amplified. To assess the configuration of immunoglobulin heavy-chain genes, Southern blotting was performed with the use of ³²P-labeled probes to the J_H region (Dako) after the digestion of DNA with *Bgl*II or *Bam*HI (New England Biolabs, Beverly, Mass.). The presence of EBV was detected on Southern blots with the use of a *Bam*HI W probe specific for EBV genomic DNA. Cytogenetic analysis was performed on cells in metaphase harvested directly from the pleural fluid. Harvesting of cells, slide making, and trypsin-Giemsa banding were performed as described previously.³³

RESULTS

Cytopathological, Genotypic, and Karyotypic Findings

The patient's pleural fluid contained numerous large plasmacytoid cells, most of which had one to three prominent nucleoli and moderate amounts of deeply basophilic cytoplasm (Fig. 1). Multinucleated cells and scattered mitotic figures were also seen. Immunohistochemical and flow-cytometric analyses revealed that these cells bound antibodies against the activation markers CD30, epithelial membrane antigen, and CD38 and failed to react with antibodies against B-cell markers (CD10, CD19, CD20, and CD79a), T-cell markers (CD3, CD5, and CD45RO), myelomonocytic markers (CD11b, CD13, CD14, and CD33), CD45, cytokeratin, or EBV latent membrane protein 1. The tumor cells also failed to express EBV-encoded RNA transcripts, as assessed by in situ hybridization.

Southern blot analysis performed with DNA prepared from the pleural fluid revealed two non-germline immunoglobulin heavy-chain bands of approximately equal intensity, a finding compatible with the presence of a single dominant lymphoid clone that had rearranged both immunoglobulin heavy-chain alleles (data not shown). No hybridizing bands were observed with a probe specific for EBV genomic sequences. Cytogenetic analysis of 10 cells in metaphase from the pleural fluid revealed the following clonal chromosomal aberrations: 47-50,XY; der(2)add(2)-(p23)add(2)(q37); add(4)(q25); add(4)(q35); -5; add(6)(q21); del(7)(q12); del(9)(p21); -10; add(11)-(q25); add(12)(q24); add(14)(q24); add(17)(p12); add(18)(q23); +19; +20; -21; -22; +mar1; and +2-5mars.

Detection of HHV-8 Latency-Associated Nuclear Antigen

To detect the presence of HHV-8-specific antigens in tumor cells from the patient's pleural fluid, indirect

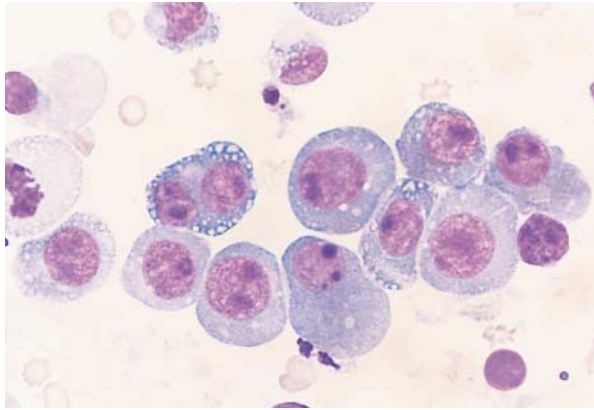


Figure 1. Cytospin Preparation of Pleural Fluid in a Patient with Primary-Effusion Lymphoma, Showing Large Plasmacytoid Tumor Cells (Wright-Giemsa Stain, $\times 1000$).

immunofluorescent staining was performed with immune serum from an HHV-8-infected patient. Antibodies from most HHV-8-infected patients react with the latency-associated nuclear-antigen complex,³⁴ which includes an HHV-8-encoded polypeptide of unknown function. Punctate nuclear staining in a pattern consistent with the distribution of latency-associated nuclear antigen was seen with immune serum in more than 90 percent of the tumor cells (Fig. 2A). A small population of cells in the effusion fluid

demonstrated diffuse immunofluorescent staining independently of the presence or absence of immune serum (Fig. 2A and 2B), a finding consistent with nonspecific binding of secondary antibody to admixed macrophages, but no punctate nuclear staining was seen when immune serum was omitted (Fig. 2B). Immune serum produced an identical pattern of punctate nuclear staining in the HHV-8-infected BC-1 cell line (Fig. 2C) and the BCBL-1 cell line, whereas no staining was seen in the EBV-infected B-cell lines IB4 and B95-8. Supernatants from the patient's pleural fluid also produced strong punctate nuclear staining in the BC-1 and BCBL-1 cell lines, indicating the presence of HHV-8 antibodies.

Ultrastructural Studies

Electron-microscopical examination of the pleural fluid revealed large tumor cells (10 to 20 μm in diameter) with prominent nucleoli, abundant rough endoplasmic reticulum and free ribosomes, and short, blunt surface projections (Fig. 3). A number of tumor cells contained herpesvirus nucleocapsids in various stages of maturation, including complete virions, approximately 100 to 150 nm in diameter, with round, electron-dense cores, as well as incomplete virions without cores. Viral particles were most numerous in cells with peripheralization of nuclear chromatin and disruption of the nuclear membrane. Occasional enveloped forms were seen budding from the nuclear membrane (Fig. 3, inset). A few viral particles were also seen within admixed benign macrophages.

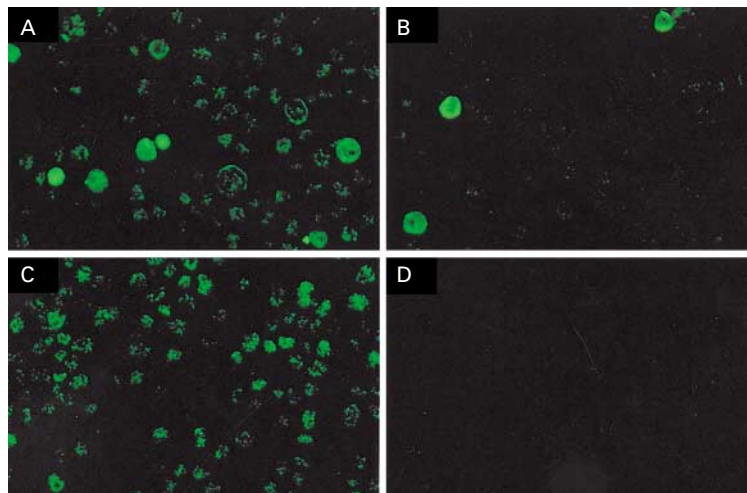


Figure 2. Expression of Latency-Associated Nuclear Antigen in Primary-Effusion Lymphoma.

Tumor cells from the patient's pleural fluid (Panels A and B) and from an HHV-8-infected cell line, BC-1 (Panels C and D), were stained with fluorescein isothiocyanate-conjugated antihuman IgG antibody after incubation with serum from an HHV-8-infected patient (Panels A and C) or fluorescein isothiocyanate-conjugated IgG antibody alone (Panels B and D). The images were obtained with the use of a confocal microscope. Because of brighter staining in the primary tumor cells, the images shown in Panels A and B were obtained with 10 percent of the light used for Panels C and D.

Detection of HHV-8 DNA in Archival Tissue Specimens

To determine the duration of HHV-8 infection in the patient, PCR was used to screen archival tissue specimens for HHV-8 genomic sequences. With the use of a primer set that is specific for HHV-8, abundant product of the expected size (233 bp) was noted after only 15 cycles of amplification of tumor DNA (Fig. 4), a result that can be explained by the presence of a large number of HHV-8 copies in the tumor-cell population due to active viral replication. Smaller amounts of HHV-8-specific product were also amplified from template DNA prepared from a lymph-node-biopsy specimen involved by Kaposi's sarcoma (May 1990), one of the post-transplantation endomyocardial-biopsy specimens with prominent plasmacytic infiltrates (October 1997), and tissue from the explanted heart (December 1989). HHV-8 sequences were not detected in an endomyocardial-biopsy specimen that had no plasmacytic infiltrates (March 1990) or in a colon-biopsy specimen obtained for evaluation of gastrointestinal bleeding (July 1997), even when DNA was subjected to 40 cycles of amplification.

DISCUSSION

Primary-effusion lymphoma, a rare, rapidly fatal neoplasm, was initially described in patients with late-stage AIDS.^{3,7,35,36} The tumor has a distinctive presentation, with malignant peritoneal, pericardial, or pleural effusions in the absence of an identifiable tumor mass or nodal involvement. It consists of large nucleolated cells with an immunoblastic or anaplastic appearance, which are positive for activation markers such as CD30 and negative for B-cell markers. An association with both EBV and HHV-8 infection has been established. Genotypic analysis invariably demonstrates immunoglobulin-gene rearrangements, confirming that these tumors originate from B cells.^{3,7,36} Karyotypes have been described for only two other primary-effusion lymphomas, both of which also had complex chromosomal aberrations without rearrangements specific for other types of lymphoma.³⁶ The tumor described here resembled those previously reported, except that it had a more plasmacytic appearance, did not express CD45, and was not associated with either EBV or HIV infection.

The dual infection of tumor cells with EBV and HHV-8 in most cases of primary-effusion lymphoma has suggested that these herpesviruses have a synergistic role and has raised questions about the relative contribution of each virus to the transformed phenotype. The absence of EBV in tumor cells from our patient suggests that HHV-8 is the critical infectious agent in the pathogenesis of primary-effusion lymphoma. The recent description of five other EBV-negative cases of primary-effusion lymphoma and the establishment of several EBV-negative cell lines from these tumors further support the key role of HHV-8.^{26,27,37-40}

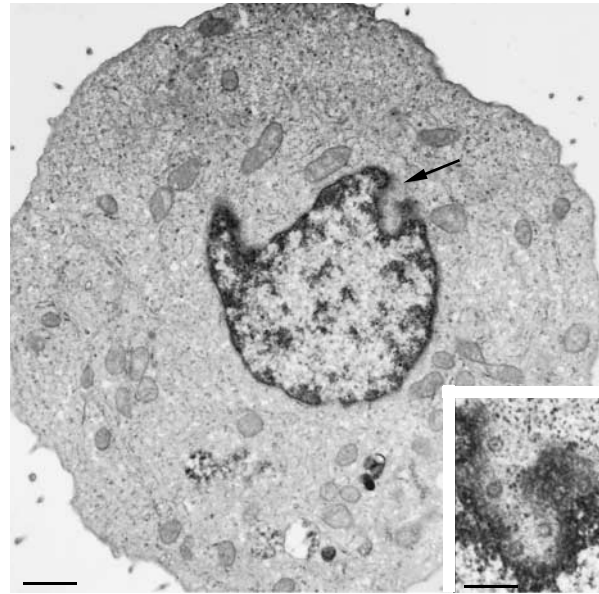


Figure 3. Electron Micrograph Showing Herpes Virions in Primary Isolates of Tumor Cells.

The tumor cells are minimally differentiated, with intranuclear nucleocapsids, including occasional enveloped forms budding from the nuclear membrane (arrow) (scale bar, 1 μ m). The inset shows herpesvirus nucleocapsids with diameters of 100 to 150 nm, which is consistent with previous findings in HHV-8-infected tumor cells (scale bar, 500 nm).

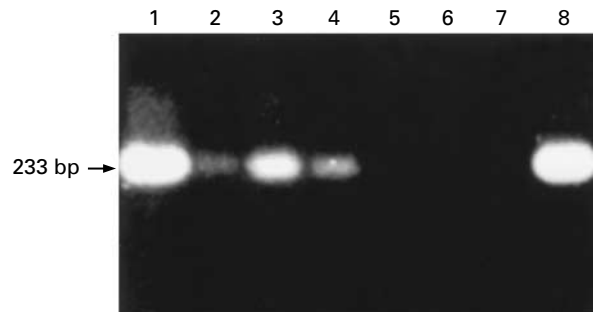


Figure 4. HHV-8 DNA in the Pleural Fluid and Archival Tissue Specimens from the Patient.

PCR analysis was performed with a primer set that amplifies a 233-bp fragment from the KS₃₃₀ region of HHV-8. Lane 1 shows the pleural-fluid sample, lane 2 an endomyocardial-biopsy specimen with persistent plasmacytic infiltrates, lane 3 a specimen from a post-transplantation lymph-node biopsy showing Kaposi's sarcoma, lane 4 tissue from the explanted heart, lane 5 an endomyocardial-biopsy specimen without plasmacytic infiltrates, lane 6 a colon-biopsy specimen, lane 7 a negative control (DNA from a large-cell lymphoma in an HIV-positive patient), and lane 8 a positive control (DNA from a previously characterized primary-effusion lymphoma).

Most cases of EBV-negative primary-effusion lymphoma have occurred in patients who were also HIV-negative, whereas primary-effusion lymphomas in HIV-positive patients are usually EBV-positive. The reason for these virologic differences is unknown. The status of HHV-8 infection in primary-effusion-lymphoma cell lines varies, although the infection is usually latent, with only a small number of cells undergoing lytic replication.^{31,41-44} We detected viral replication in freshly isolated tumor cells, as has been found in primary-effusion-lymphoma cell lines passaged in mice.²⁹

Detection of HHV-8 DNA sequences in the explanted heart of our patient conclusively demonstrates that infection preceded cardiac transplantation. Whether viral infection contributed in any way to the development of dilated cardiomyopathy is uncertain. No link has been established between HHV-8 infection and cardiac disease, and it is possible that the positive PCR result reflected the presence of HHV-8–infected lymphocytes or monocytes in the tissue. The source of HHV-8 infection in our patient is unknown, but he was from Haiti, where the prevalence of antibodies against HHV-8 is relatively high. Recent studies indicate that sexual transmission is the predominant mode of spread of HHV-8.^{45,46} In one series in Italy, a region where HHV-8 infection is relatively common, Kaposi's sarcoma appeared to result from an infection acquired before solid-organ transplantation in 10 of 11 patients; as in our patient, lesions often arose within six months after transplantation.⁴⁷ Together, these observations suggest that prior viral infection is the principal cause of HHV-8–associated neoplasia in recipients of solid-organ transplants.

Primary-effusion lymphoma is typically highly aggressive and refractory to therapy. It would be useful to identify patients who are at high risk for this tumor, since therapy with antiviral agents might suppress lymphomagenesis.³¹ The HHV-8 genome encodes genes homologous to several cellular proto-oncogenes implicated in B-cell lymphomagenesis, including *Bcl-2* and cyclin D, as well as growth factors such as interleukin-6, all of which may contribute to the proliferation and survival of infected B cells.^{48,49} The presence of persistent hypergammaglobulinemia and polyclonal plasmacytic infiltrates in the myocardium and other tissues in our patient suggests that widespread B-cell hyperplasia preceded the development of lymphoma.

Supported by grants from the National Cancer Institute (CA66849, CA67380, and CA73580) and the Cancer Research Institute.

We are indebted to Dr. Geraldine Pinkus for assistance with the immunohistochemical and *in situ* hybridization studies; to Dr. Janina Longtine, Dr. Frank Kuo, and Genya Milman for assistance with the Southern blot studies; to Drs. Richard Benjamin and David Dorfman for assistance with the flow-cytometric studies; and to Thomas Morgan for assistance with the karyotypic analysis.

REFERENCES

- Cesarman E, Knowles DM. Kaposi's sarcoma-associated herpesvirus: a lymphotropic human herpesvirus associated with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castlemans disease. *Semin Diagn Pathol* 1997;14:54-66. [Erratum, *Semin Diagn Pathol* 1997;14:161-2.]
- Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994; 266:1865-9.
- 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* 1995;332:1186-91.
- Karcher DS, Alkan S. Herpes-like DNA sequences, AIDS-related tumors, and Castlemans disease. *N Engl J Med* 1995;333:797-8.
- Oskenhendler E, Duarte M, Soulier J, et al. Multicentric Castlemans disease in HIV infection: a clinical and pathological study of 20 patients. *AIDS* 1996;10:61-7.
- Soulier J, Grollet L, Oskenhendler E, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castlemans disease. *Blood* 1995;86:1276-80.
- Karcher DS, Alkan S. Human herpesvirus-8-associated body cavity-based lymphoma in human immunodeficiency virus-infected patients: a unique B-cell neoplasm. *Hum Pathol* 1997;28:801-8.
- Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infection. *N Engl J Med* 1995;332:1181-5.
- Marchioli CC, Love JL, Abbott LZ, et al. Prevalence of human herpesvirus 8 DNA sequences in several patient populations. *J Clin Microbiol* 1996;34:2635-8.
- Parravicini C, Corbellino M, Paulli M, et al. Expression of a virus-derived cytokine, KSHV vIL-6, in HIV-seronegative Castlemans disease. *Am J Pathol* 1997;151:1517-22.
- Chadburn A, Cesarman E, Nador RG, Liu YF, Knowles DM. Kaposi's sarcoma-associated herpesvirus sequences in benign lymphoid proliferations not associated with human immunodeficiency virus. *Cancer* 1997;80: 788-97.
- Kedda MA, Margolius L, Kew MC, Swanepoel C, Pearson D. Kaposi's sarcoma-associated herpesvirus in Kaposi's sarcoma occurring in immunosuppressed renal transplant recipients. *Clin Transplant* 1996;10:429-31.
- Alkan S, Karcher DS, Ortiz A, Khalil S, Akhtar M, Ali MA. Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus in organ transplant patients with immunosuppression. *Br J Haematol* 1997;96:412-4.
- Cathomas G, McGandy CE, Terracciano LM, et al. Nachweis von Herpes-Virus ähnlicher DNA in den verschiedenen Formen des Kaposi Sarkoms aber nicht in anderen mesenchymalen Tumoren oder entzündlichen Veränderungen der Haut. *Verh Dtsch Ges Pathol* 1996;80:318-21.
- Boshoff C, Talbot S, Kennedy M, O'Leary J, Schulz T, Chang Y. HHV8 and skin cancers in immunosuppressed patients. *Lancet* 1996;347: 338-9. [Erratum, *Lancet* 1996;348:138.]
- Cathomas G, Tamm M, McGandy CE, et al. Transplantation-associated malignancies: restriction of human herpes virus 8 to Kaposi's sarcoma. *Transplantation* 1997;64:175-8.
- Foreman K, Bonish B, Nickoloff B. Absence of human herpesvirus 8 DNA sequences in patients with immunosuppression-associated dermatofibromas. *Arch Dermatol* 1997;133:108-9.
- Lebbe C, Tatoud R, Morel P, et al. Human herpesvirus 8 sequences are not detected in epithelial tumors from patients receiving transplants. *Arch Dermatol* 1997;133:111.
- Takata M, Hatta N, Takehara K, Fujiwara H. Absence of human herpesvirus-8 DNA in angiosarcomas and other skin tumours in immunocompetent patients, and in graft-versus-host disease in the immunosuppressed recipients of bone marrow transplants. *Br J Dermatol* 1997;137:156-7.
- Dupin N, Gorin I, Escande JP, et al. Lack of evidence of any association between human herpesvirus 8 and various skin tumors from both immunocompetent and immunosuppressed patients. *Arch Dermatol* 1997;133:537.
- Luppi M, Barozzi P, Maiorana A, et al. Human herpesvirus-8 DNA sequences in human immunodeficiency virus-negative angioimmunoblastic lymphadenopathy and benign lymphadenopathy with giant germinal center hyperplasia and increased vascularity. *Blood* 1996;87:3903-9.
- Pawson R, Catovsky D, Schulz TF. Lack of evidence of HHV-8 in mature T-cell lymphoproliferative disorders. *Lancet* 1996;348:1450-1.
- Dupin N, Franck N, Calvez V, et al. Lack of evidence of human herpesvirus 8 DNA sequences in HIV-negative patients with various lymphoproliferative disorders of the skin. *Br J Dermatol* 1997;136:827-30.
- Pastore C, Gloghini A, Volpe G, et al. Distribution of Kaposi's sarcoma herpesvirus sequences among lymphoid malignancies in Italy and Spain. *Br J Haematol* 1995;91:918-20.
- Gessain A, Briere J, Angelin-Duclos C, et al. Human herpes virus 8 (Kaposi's sarcoma herpes virus) and malignant lymphoproliferations in

- France: a molecular study of 250 cases including two AIDS-associated body cavity based lymphomas. *Leukemia* 1997;11:266-72.
26. Cesarman E, Nador RG, Aozasa K, Delsol G, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus in non-AIDS related lymphomas occurring in body cavities. *Am J Pathol* 1996;149:53-7.
27. Said JW, Tasaka T, Takeuchi S, et al. 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. *Blood* 1996;88:3124-8.
28. Ibrahim RE, Teich D, Smith BR, Antin J, Oliver AP, Weinberg DS. Flow cytometric surface light chain analysis of lymphocyte-rich effusions: a useful adjunct to cytologic diagnosis. *Cancer* 1989;70:2024-9. [Erratum, *Cancer* 1989;64:1436.]
29. Said W, Chien K, Takeuchi S, et al. Kaposi's sarcoma-associated herpesvirus (KSHV or HHV8) in primary effusion lymphoma: ultrastructural demonstration of herpesvirus in lymphoma cells. *Blood* 1996;87:4937-43.
30. Cesarman E, Moore PS, Rao PH, Inghirami G, Knowles DM, Chang Y. In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. *Blood* 1995;86:2708-14.
31. Renne R, Zhong W, Herndier B, et al. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. *Nat Med* 1996;2:342-6.
32. Bigoni B, Dolcetti R, de Lellis L, et al. Human herpesvirus 8 is present in the lymphoid system of healthy persons and can reactivate in the course of AIDS. *J Infect Dis* 1996;173:542-9.
33. Fletcher JA, Kozakewich HP, Hoffer FA, et al. Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumors. *N Engl J Med* 1991;324:436-43.
34. Rainbow L, Platt GM, Simpson GR, et al. The 222- to 234-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. *J Virol* 1997;71:5915-21.
35. Jones D, Weinberg DS, Pinkus GS, Renshaw AA. Cytologic diagnosis of primary serous lymphoma. *Am J Clin Pathol* 1996;106:359-64.
36. Ansari MQ, Dawson DB, Nador R, et al. Primary body cavity-based AIDS-related lymphomas. *Am J Clin Pathol* 1996;105:221-9.
37. Hsi ED, Foreman KE, Duggan J, et al. Molecular and pathologic characterization of an AIDS-related body cavity-based lymphoma, including ultrastructural demonstration of human herpesvirus-8: a case report. *Am J Surg Pathol* 1998;22:493-9.
38. Boshoff C, Gao S-J, Healy LE, et al. Establishing a KSHV+ cell line (BCP-1) from peripheral blood and characterizing its growth in Nod/SCID mice. *Blood* 1998;91:1671-9.
39. Arvanitakis L, Mesri EA, Nador RG, et al. Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. *Blood* 1996;88:2648-54.
40. Gao SJ, Kingsley L, Li M, et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nat Med* 1996;2:925-8.
41. Miller G, Heston L, Grogan E, et al. Selective switch between latency and lytic replication of Kaposi's sarcoma herpesvirus and Epstein-Barr virus in dually infected body cavity lymphoma cells. *J Virol* 1997;71:314-24.
42. Decker LL, Shankar P, Khan G, et al. The Kaposi sarcoma-associated herpesvirus (KSHV) is present as an intact latent genome in KS tissue but replicates in the peripheral blood mononuclear cells of KS patients. *J Exp Med* 1996;184:283-8.
43. Renne R, Lagunoff M, Zhong W, Ganem D. The size and conformation of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in infected cells and virions. *J Virol* 1996;70:8151-4.
44. Horenstein MG, Nador RG, Chadburn A, et al. Epstein-Barr virus latent gene expression in primary effusion lymphomas containing Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8. *Blood* 1997;90:1186-91.
45. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. *N Engl J Med* 1998;338:948-54.
46. Goedert JJ, Kedes DH, Ganem D. Antibodies to human herpesvirus 8 in women and infants born in Haiti and the USA. *Lancet* 1997;349:1368.
47. Parravicini C, Olsen SJ, Capra M, et al. Risk of Kaposi's sarcoma-associated herpes virus transmission from donor allografts among Italian posttransplant Kaposi's sarcoma patients. *Blood* 1997;90:2826-9.
48. Burger R, Neipel F, Fleckenstein B, et al. Human herpesvirus type 8 interleukin-6 homologue is functionally active on human myeloma cells. *Blood* 1998;91:1858-63.
49. Ganem D. KSHV and Kaposi's sarcoma: the end of the beginning? *Cell* 1997;91:157-60.