

ASSOCIATION OF EPSTEIN-BARR VIRUS WITH LEIOMYOSARCOMAS IN YOUNG PEOPLE WITH AIDS

KENNETH L. McCLAIN, M.D., PH.D., CHARLES T. LEACH, M.D., HAL B. JENSON, M.D., VIJAY V. JOSHI, M.D., PH.D., BRAD H. POLLOCK, M.P.H., PH.D., RICHARD T. PARMLEY, M.D., FREDERICK J. DiCARLO, M.D., ELLEN GOULD CHADWICK, M.D., AND SHARON B. MURPHY, M.D.

Abstract Background. Children with the acquired immunodeficiency syndrome (AIDS) have an unusually high incidence of smooth-muscle tumors (leiomyomas and leiomyosarcomas) in addition to malignant lymphomas. We tested the hypothesis that the smooth-muscle tumors in these children are associated with the Epstein-Barr virus (EBV).

Methods. Tissue specimens of five leiomyosarcomas and two leiomyomas from five children and one young man with AIDS were studied for evidence of the human immunodeficiency virus (HIV) and EBV by in situ hybridization and quantitative polymerase chain reaction (PCR). Comparison specimens included samples of leiomyosarcoma and leiomyoma from HIV-negative children. EBV clonality of leiomyosarcomas was determined by Southern blot analysis with oligonucleotide probes for EBV terminal-repeat fragments. Tumor specimens were tested by immunoperoxidase staining for infiltration by B lymphocytes and expression of the EBV receptor. Serologic testing for EBV was performed.

Results. In situ hybridization showed EBV genomes in all muscle cells of the five leiomyosarcomas and the two leiomyomas from the six HIV-infected patients. Quantitative PCR demonstrated strikingly high levels of EBV in tumor tissue, with as many as 4.3 genome copies per cell. Two colonic leiomyosarcomas obtained from different sites at different times from one patient contained different episomal EBV clones, signifying the presence of distinct monoclonal EBV-related tumors. We found biclonal EBV infection in the leiomyosarcoma of another patient. No EBV was detected in normal muscle or tumor specimens from HIV-negative patients. Immunostaining for the EBV receptor was strongly positive in six of the seven leiomyomas and leiomyosarcomas from the patients with AIDS.

Conclusions. EBV can infect smooth-muscle cells, at least in patients with AIDS, and it may contribute to the pathogenesis of leiomyomas and leiomyosarcomas in patients with AIDS. EBV seems to play no part in smooth-muscle tumors in HIV-negative patients. (N Engl J Med 1995;332:12-8.)

ADULTS with the acquired immunodeficiency syndrome (AIDS) have an increased susceptibility to Kaposi's sarcoma and B-cell lymphomas, especially central nervous system lymphoma.¹⁻³ Although the incidence of these cancers in children with AIDS has not yet reached that seen in adults, the frequent occurrence of leiomyosarcomas has been unexpected and intriguing. Leiomyosarcoma is an extremely rare tumor in children; its annual incidence is less than 2 cases per 10 million children.⁴ The five leiomyosarcomas and two leiomyomas in the six patients with human immunodeficiency virus (HIV) infection described in this report (three of whom have been described previously) plus the seven cases reported by others amount to a frequency much higher than expected among the approximately 5000 children with AIDS in the United States.⁵⁻¹⁰ The predilection for sarcomatous involvement of the gastrointestinal tract and lungs is striking but unexplained. An intranodal leiomyoma has been reported in one adult patient with AIDS.¹¹ A role has been suggested for the Epstein-Barr virus (EBV) in spindle-cell sarcomas after liver transplantation.¹² To test the hypothesis that EBV or HIV may be a cofactor for the soft-tissue

tumors of patients with AIDS, we evaluated tumor cells by in situ hybridization and quantitative polymerase chain reaction (PCR) for the presence of HIV and EBV and the clonality of EBV.

METHODS

Patients

Three children and one young man who had AIDS and leiomyosarcoma, one child with AIDS and a leiomyoma, and a sixth child with AIDS and both a leiomyosarcoma and a leiomyoma were identified from 1988 through 1993 (Table 1). These six patients represent all the cases of smooth-muscle tumors reported to the Pediatric AIDS Lymphoma Network. For comparison, samples of leiomyosarcoma from three HIV-negative children were obtained from the Pediatric Oncology Group Protocol 8653, a study of soft-tissue sarcomas. These three patients were similar to the five patients with AIDS and leiomyosarcoma with regard to age, sex, and race or ethnic group. Four samples of leiomyoma from HIV-negative children, representing all the cases of leiomyoma seen at Texas Children's Hospital over a period of 40 years, also served as comparison specimens. Tumor tissue suitable for in situ hybridization was available from all patients. Samples of tumor, peripheral-blood mononuclear cells, bone marrow mononuclear cells, and plasma from the HIV-positive patients were tested for EBV and HIV by quantitative PCR. Three tumor samples from two HIV-positive patients were evaluated for EBV clonality.

EBV Serologic Testing

Anti-EBV antibodies in plasma were measured by standard immunofluorescence methods.¹³ These assays included tests for IgG, IgM, and IgA antibodies to viral capsid antigen (VCA-IgG, VCA-IgM, and VCA-IgA, respectively); IgG antibodies to early antigens, including both the diffuse and the restricted components; and IgG antibodies to Epstein-Barr nuclear antigen (EBNA).

In Situ Hybridization

Sections of tumors were prepared on glass slides by the methods of Chang et al. and were hybridized with biotinylated oligonucleotides complementary to three regions of the EBV *EBER-1* (*EBER*) gene or

From the Department of Pediatrics, Baylor College of Medicine, Houston (K.L.M.); the Department of Pediatrics, University of Texas Health Science Center at San Antonio (C.T.L., H.B.J.); the Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, N.C. (V.V.J.); the Department of Pediatrics, University of Florida College of Medicine, Gainesville (B.H.P.); Carolinas Medical Center, Charlotte, N.C. (R.T.P.); the Department of Pathology, Children's Hospital of New Jersey, Newark (F.J.D.); and the Department of Pediatrics, Northwestern University Medical School and the Children's Memorial Hospital, Chicago (E.G.C., S.B.M.). Address reprint requests to Dr. McClain at the Texas Children's Hospital, Children's Cancer Center MC 3-3320, 6621 Fannin St., Houston, TX 77030.

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Table 1. Clinical Data on the Patients with Smooth-Muscle Tumors.

PATIENT NO.	RACE OR ETHNIC GROUP	AGE (YR) AT TUMOR DIAGNOSIS/SEX	TUMOR SITE	TUMOR TYPE	AGE (YR) AT DIAGNOSIS OF AIDS	ROUTE OF HIV TRANSMISSION	CD4+ COUNT (CELLS/MM ³) AT TUMOR DIAGNOSIS
HIV-positive							
1	Hispanic	8/F	Lung	Leiomyosarcoma	4	Perinatal transfusion	3
2	Hispanic	4/F	Colon	Leiomyoma			
3	Black	7/F	Stomach	Leiomyosarcoma	2	Perinatal	43
4	Black	7/F	Intestine	Leiomyosarcoma	2	Perinatal	9
4	Hispanic	24/M	Liver	Leiomyosarcoma	18	Transfusion	20
5	White	5/F	Colon	Leiomyosarcoma	1	Perinatal	330
6	Black	4/M	Lung	Leiomyoma	4	Perinatal transfusion	6
HIV-negative							
7	Black	7/M	Rectum	Leiomyosarcoma	—	—	—
8	White	14/F	Stomach	Leiomyosarcoma	—	—	—
9	White	8/F	Labia majora	Leiomyosarcoma	—	—	—
10	White	12/F	Stomach	Leiomyoma	—	—	—
11	Hispanic	5/M	Ear	Leiomyoma	—	—	—
12	White	3/F	Ileocecum	Leiomyoma	—	—	—
13	White	3/M	Finger	Leiomyoma	—	—	—

the HIV *gag* gene.¹⁴ The genes were detected with the Detek Hrp Signal Generating System (Enzo Diagnostics, Farmingdale, N.Y.).

Quantitative PCR

Tumor samples, peripheral-blood mononuclear cells, and bone marrow mononuclear cells were obtained from Patient 4 at the time of tumor diagnosis and from Patient 5 during the period from 0.1 to 1.1 years after diagnosis. Plasma samples obtained from Patient 4 4.6 to 1.5 years before tumor diagnosis were available. Plasma samples obtained from Patient 6 at the time of tumor diagnosis and samples of peripheral-blood mononuclear cells and plasma from Patient 7 obtained 2.4 years after diagnosis also were tested.

Mononuclear cells were collected from the peripheral-blood samples and bone marrow aspirates by Ficoll-Hypaque density-gradient centrifugation. For PCR, cell samples were lysed¹⁵ and plasma was heated at 70°C for 45 seconds.¹⁶ Tumor specimens required DNA extraction by standard methods.

PCR was performed in a total volume of 20 μ l with 5 μ l of sample containing 100,000 cells, 0.67 μ g of tumor DNA, or 1 μ l of plasma diluted in standard PCR buffer. Primers W-1 (5'GTTTCGCGTTGC-TAGGCCACC3') and W-2b (5'TGGCGCTCTGATGCGACCAG3'), which amplify a 140-base-pair portion of the *Bam*HI W fragment of EBV, were used to amplify EBV.^{17,18} Each PCR run included a set of copy-number controls consisting of 1 to 1000 copies of a plasmid containing the amplified region and converted to linear form, diluted in a background of 100,000 lysed, uninfected H9 cells. As an internal control, primers PCO4 and GH20, which amplify a conserved β -globin sequence, were included in each reaction.¹⁹ The cycling conditions were 94°C for 3 minutes, 40 cycles at 68°C for 2 minutes and 94°C for 1 minute, and a final incubation at 68°C for 10 minutes. Hybridization was performed with EBV probe *Bam*HI W¹⁸ and β -globin probe 19A¹⁹ labeled with γ -³²P]ATP.

To amplify HIV, primers SK38 and SK39 were used.¹⁵ A plasmid containing the HIV-1 *gag* region and converted to linear form was used as a copy-number control.²⁰ The cycling conditions were identical to those used to amplify EBV, except that annealing and extension were performed at 65°C. Radiolabeled probes SK19 (HIV) and 19A (β -globin) were used for hybridization.

Radioactivity in each band was measured with a Betascope imager (Betagen, Framingham, Mass.) and quantitated with a standard curve for each PCR run. The β -globin signal was used to correct the PCR signal for EBV or HIV in cell samples to correspond to 100,000 cell genomes. An average of six repeats of the *Bam*HI W fragment was assumed for each EBV genome.²¹ Appropriate dilutions were made in all instances so that the number of copies of EBV or HIV in each sample was well within the standard curve.

EBV Clonality

Sufficient DNA to permit Southern blot analysis for EBV clonality was available from a single tumor-biopsy specimen from Patient 4

and from two colon-tumor specimens obtained at different times from Patient 5. Intracellular DNA was digested with *Bam*HI, analyzed by Southern blotting, probed with a fragment of *Eco*RI I located near the terminal repeat at one end of the genome, and then stripped and probed with a fragment of *Xho*I located near the opposite end of the genome.²²

Immunostaining for EBV Receptor and Cell Phenotype

Tissue sections were deparaffinized and immunostained²³ with mouse monoclonal anti-CD21 antibodies to the human EBV receptor (CD21)²⁴ and anti-CD20 antibodies to the human B-cell antigen (CD20) (Becton Dickinson, San Jose, Calif.) with Vectastain Elite ABC-DAB Substrate kits (Vector Laboratories, Burlingame, Calif.). Two independent observers agreed on the grade of staining in all cases.

RESULTS

Pathological Characterization of Smooth-Muscle Tumors

On light-microscopical examination, the leiomyosarcomas had a uniformly dense cellularity. The fusiform cells of the tumors were arranged in fascicles; their hyperchromatic nuclei had up to five mitotic figures per high-power field. Muscle-specific stains for actin and desmin were uniformly positive. The leiomyomas were composed of fusiform-to-spindle-shaped cells, with few mitotic figures or none.

Immunohistochemical stains were positive for anti-muscle actin and desmin in all tumors. Electron-microscopical analysis of specimens from Patients 2, 4, and 5 (not shown) showed thin intracytoplasmic filaments with focal densities, abundant micropinocytotic vesicles, thin but definite external laminae, and folded nuclei. These features further confirmed the smooth-muscle origin of the tumor cells.

EBV Serologic Testing

Plasma samples obtained at the time of tumor diagnosis were available from three HIV-positive patients and one HIV-negative patient. All four patients had evidence of past EBV infection, as indicated by the presence of VCA-IgG antibodies and the absence of VCA-IgM antibodies. VCA-IgA antibodies were found at the minimal detectable dilution (1:8) in Patient 5, who had high VCA-IgG antibody titers (1:1280). Low levels of

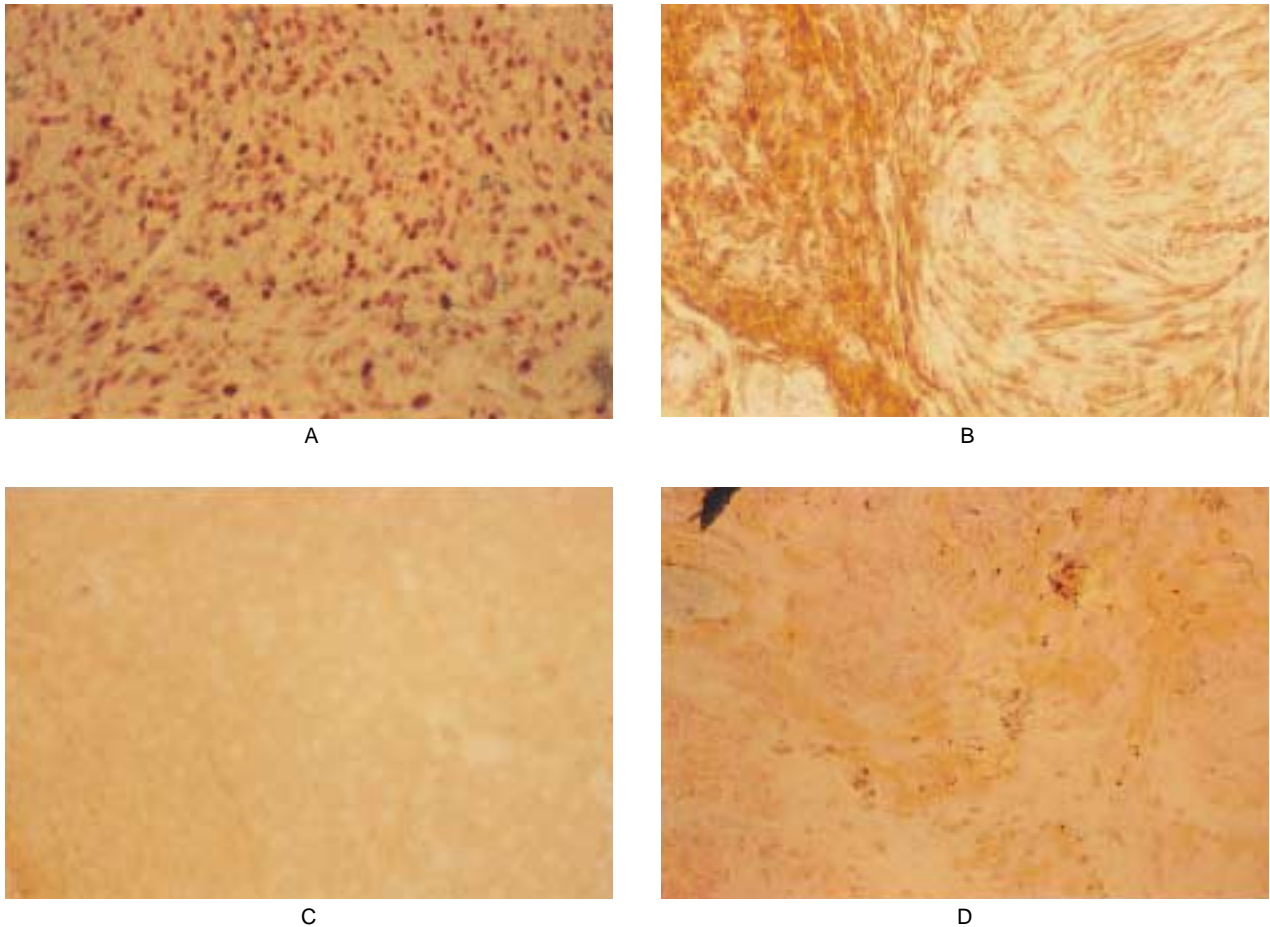


Figure 1. Photomicrographs of Tumor Specimens Studied to Detect EBV.

Panel A ($\times 630$) shows in situ hybridization of a leiomyosarcoma specimen from an HIV-positive patient (Patient 1). When tested with the *EBER* probe, the sample shows bright-red nuclear staining, indicating prominent hybridization of the biotinylated probe. Panel B ($\times 250$) shows immunoperoxidase staining of the tissue from Patient 1 with antibody to the EBV receptor (CD21). The golden-brown precipitate observed on staining with 3,3'-diaminobenzidine (DAB) peroxidase reveals masses of tumor cells that bound the CD21 antibody. Under identical conditions, a pan-B-cell antibody, CD20, did not react with the tissue. Panel C ($\times 630$) shows in situ hybridization of a leiomyoma specimen from an HIV-negative patient (Patient 10). When this sample was tested with the *EBER* probe as in Panel A, there was no detectable hybridization of the probe. Panel D ($\times 250$) shows immunoperoxidase staining of the tissue from Patient 10 with antibody to CD21. There are moderate numbers of golden-brown precipitates in the muscle fibers on staining with DAB peroxidase.

IgG antibodies to the restricted component of early antigen were found in Patients 4 and 7; Patient 5 had a high level of IgG antibodies to the diffuse component of early antigen (1:320). Two HIV-positive patients and the HIV-negative patient had antibodies to Epstein-Barr nuclear antigen.

In Situ Hybridization

None of the tumors from the HIV-positive or HIV-negative patients had evidence of intracellular HIV infection on in situ hybridization. In contrast, the five leiomyosarcoma specimens and two leiomyoma specimens from the patients with AIDS had strong nuclear staining for EBV in essentially all tumor cells (Fig. 1A). Treatment of the tissue sections with RNase-T1 prevented hybridization of the *EBER* probes (data not shown). Normal smooth muscle in the HIV-positive and HIV-negative patients was uniformly negative for

EBER hybridization, indicating the absence of EBV. Nor did the three leiomyosarcomas and the four leiomyomas from the HIV-negative patients have evidence of *EBER* on in situ hybridization (Fig. 1C).

Quantitative PCR

Available samples of tumor, peripheral-blood mononuclear cells, bone marrow mononuclear cells, and plasma were analyzed by quantitative PCR for HIV and EBV (Table 2). Strikingly high levels of EBV were found in the two initial tumor samples: 282,422 and 426,455 copies per 100,000 cells (from Patients 4 and 5, respectively). If all the tumor cells were uniformly infected with EBV (as indicated by in situ hybridization), the average number of copies of the EBV genome per cell in the two samples would be 2.8 and 4.3. The levels of EBV in serial specimens of peripheral-blood mononuclear cells (range, 0 to 436,461 copies per 100,000

Table 2. PCR Quantitation of Copies of the EBV and HIV Genomes in Samples of Tumor, Plasma, Peripheral-Blood Mononuclear Cells, and Bone Marrow Mononuclear Cells.

TIME SAMPLE OBTAINED (YR)*	CONCENTRATION OF EBV				CONCENTRATION OF HIV			
	TUMOR	PERIPHERAL BLOOD	BONE MARROW	PLASMA	TUMOR	PERIPHERAL BLOOD	BONE MARROW	PLASMA
	copies/100,000 cells			copies/ml	copies/100,000 cells			copies/ml
HIV-positive								
Patient 4								
-4.6	—	—	—	54	—	—	—	0
-4.2	—	—	—	25	—	—	—	0
-3.7	—	—	—	114	—	—	—	0
-1.5	—	—	—	173	—	—	—	0
0	282,422	0	1	16,740	4	0	9	1437
+0.2	—	2	—	12,342	—	31	—	659
Patient 5								
+0.1	426,455	3,584	—	6,315	5	21	—	0
+0.2	—	29,966	—	321	—	28	—	0
+0.3	230,444	—	—	—	45	—	—	—
+0.5	—	436,461	—	7,104	—	61	—	0
+0.6	—	5,219	—	9,680	—	18	—	0
+0.8	—	4,619	—	12,440	—	37	—	0
+1.1	170,456	—	—	—	14	—	—	—
Patient 6								
0	—	—	—	3,973	—	—	—	640
HIV-negative								
Patient 7								
+2.4	—	66	—	0	—	0	—	0

*Minus and plus signs indicate numbers of years before and after the diagnosis, respectively.

cells) and plasma (range, 0 to 16,740 copies per milliliter) varied and did not show a discernible trend (Table 2).

The levels of HIV in the two tumor specimens at or near the time of tumor diagnosis were almost negligible (4 and 5 copies per 100,000 cells). HIV was detectable at low levels in subsequent specimens from Patient 5 (Table 2).

EBV Clonality

EBV clonality was assessed in tumors from the two patients for whom there were adequate tissue samples (Fig. 2 and 3). Identical results were obtained with the *EcoRI* I and the *XhoI* a terminal probes; there was no evidence of EBV integration into the cell genome. Both the Raji and the B95-8 cell lines showed the expected monoclonal EBV bands; replicating forms of EBV were also found in B95-8 cells. Both tumor samples from Patient 5 yielded a single band on the Southern blot assay, indicating the presence of monoclonal EBV, but the differing sizes of these terminal-repeat bands indicated that there were separate EBV infections in each tumor. The tumor from Patient 4 had EBV genomes from two clones in approximately equal proportions, a finding consistent with either dual EBV infection or the presence of mixed monoclonal infections in equal numbers.

Immunostaining for EBV Receptor and Cell Phenotype

Cells in all the leiomyosarcomas and leiomyomas from the HIV-positive and HIV-negative patients were positive for the EBV receptor (CD21) on immunostaining (Table 3). Six of the seven tissue samples from the HIV-positive patients were strongly positive for the

EBV receptor (Fig. 1B). The samples of leiomyosarcoma and leiomyoma tissue from all seven HIV-negative patients were also positive for CD21, but generally at a lower intensity than that of the tumor samples from the HIV-positive patients (Fig. 1D). Normal smooth muscle and normal striated muscle had some immunostaining with the CD21 antibody. None of the muscle tissues reacted with the pan-B-cell antibody (CD20). Tonsil epithelium reacted strongly with both CD20 and CD21.

DISCUSSION

EBV has been intimately associated, though not necessarily in a causal fashion, with endemic (African) Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, and the B-cell lymphomas that arise in organ-transplant recipients and patients with immunodeficiency disorders, including AIDS.^{25,26} Gastric carcinoma, which is morphological-

ly similar to nasopharyngeal carcinoma, has also been found to harbor EBV, particularly in patients who have a prominent lymphoid infiltration in the tumor stroma.^{27,28} There is a single report of the presence of EBV DNA in striated muscle²⁹; muscle-biopsy specimens from 8 of 86 patients with the postviral fatigue syndrome contained 3 to 50 copies of EBV DNA per cellular equivalent of genomic DNA, as judged by Southern blot hybridization.

We found evidence of EBV infection in five leiomyosarcomas and two leiomyomas from six HIV-infected patients, but not in smooth-muscle tumors from HIV-negative patients. All the tumors we studied, regardless of source, contained the CD21 EBV receptor. Our results support the hypothesis that EBV has an etiologic role in the soft-tissue tumors that arise in patients with AIDS. EBV may contribute to smooth-muscle tumorigenesis in other immunosuppressed states as well.¹²

The results of EBV serologic testing in the four patients tested were consistent with past EBV infection. There was no specific evidence of acute infection or of the characteristic profile of anti-EBV antibodies found in patients with nasopharyngeal carcinoma or Burkitt's lymphoma.¹³

The EBV receptor (CD21) may be a prerequisite for EBV infection of smooth-muscle cells. However, some authors suggest that cell fusion with EBV-infected lymphocytes is the route of viral entry into nonlymphoid cells.³⁰ We found no evidence of *EBER*-positive cells or lymphocytic infiltrates in tissue surrounding the tumors of the HIV-positive patients. If fusion between EBV-infected lymphocytes and muscle cells had indeed occurred, it must have been at such an early stage of

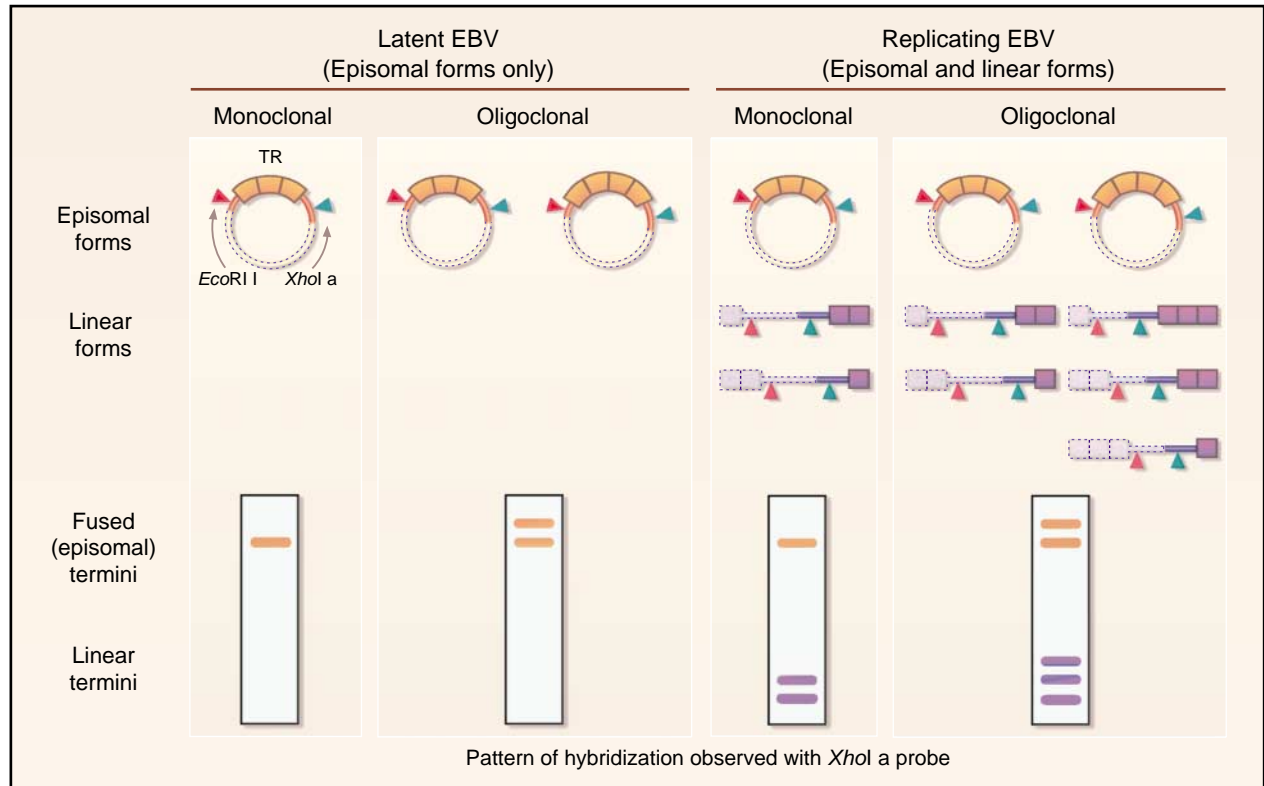


Figure 2. Determination of Clonality of EBV Infection.

The EBV genome exists in two forms, circular (episomal) and linear. In latently infected cells EBV is present only as episomes. During lytic viral replication, leading to cell death, EBV replicates with linear intermediates formed by the random cleavage between terminal repeats (TR) of the viral genome. The random cleavage produces linear EBV DNA genomes containing different numbers of terminal repeats at the ends of the linear genomes. After digestion of EBV DNA with an enzyme (e.g., *Bam*HI) that does not cut the genome within the terminal repeats, fragments containing the terminal repeats can be detected in a Southern blot assay using specific probes for either end of the genome, such as *Eco*RI I (red arrowheads) near the left-hand terminus of the genome or *Xho*I a (green arrowheads) near the right-hand terminus. In this example, the fragments of EBV DNA detected by the *Xho*I a probe are shown in solid colors; undetected EBV DNA is shown in dotted outline. (The converse would be true if the *Eco*RI I probe were used.) In monoclonal EBV infection, the fused termini of the episomes are present in a single size (usually 8 to 23 kilobase pairs); multiple sizes indicate oligoclonality or polyclonality, suggesting multiple EBV infections. The presence of a ladder of smaller terminal digestion fragments of the linear genomes indicates viral replication.

tumor development that we could not detect it. The EBV receptor has been identified on striated-muscle and other cells.³⁰

We found higher levels of the EBV receptor on tumor cells from HIV-infected patients than on tumor cells from HIV-negative patients or in normal smooth muscle (Table 3). This result suggests that perturbation of the immune system in AIDS can increase production of the EBV receptor. It is also possible that EBV infection itself causes increased expression of the receptor. It is not known whether the identical EBV receptor is found on smooth-muscle cells, epithelial cells, and B lymphocytes. Both the reactivity of the smooth-muscle tumors with a monoclonal antibody that detects the receptor on B cells and the presence of EBV in the tumor strongly suggest a biologically relevant association between the EBV receptor in smooth muscle and the virus.

Adequate amounts of specimens for quantitative PCR were available from three HIV-positive patients and one HIV-negative patient. The extraordinarily high copy numbers of EBV in the muscle-tumor cells

from HIV-infected patients are consistent with the results of in situ hybridization, which showed EBV in all tumor cells. Burkitt's lymphoma cells also have high copy numbers of EBV.³¹ High levels of free EBV were detected in the plasma of all three HIV-infected patients who were tested. The finding of 16,740 and 3973 genome copies per milliliter of plasma at the time of tumor diagnosis in Patients 4 and 6, respectively, and 6315 genome copies per milliliter of plasma six weeks after diagnosis in Patient 5 indicates the high level of EBV replication in these patients. The negligible levels of HIV in tumor cells at or near the time of diagnosis in Patients 4 and 5 (4 and 5 genome copies per 100,000 tumor cells, respectively) are also consistent with the negative results of in situ hybridization for HIV.

We cannot tell whether EBV infected the smooth-muscle cells before they were transformed into leiomyosarcomas or whether EBV is causally related to the malignant transformation of the smooth-muscle cells. The high levels of EBV are indirect evidence of a biologically relevant association between the virus and the

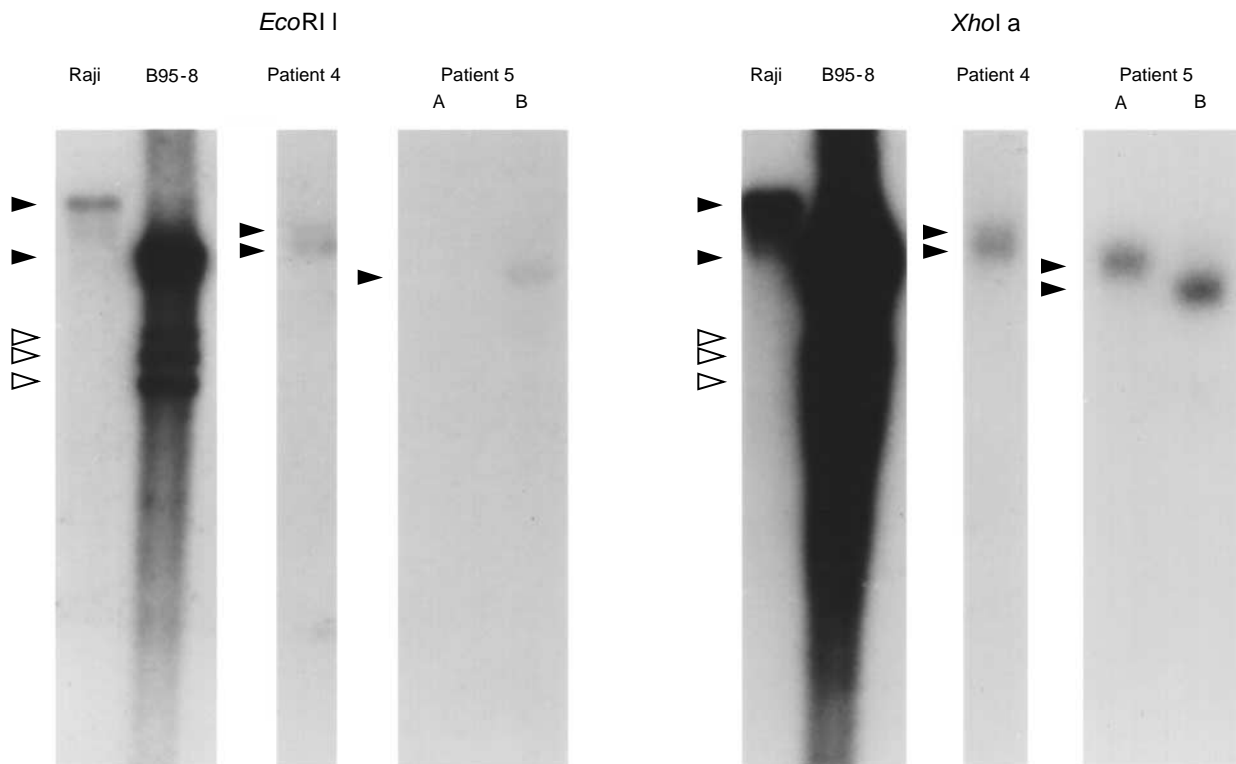
Table 3. Results of Tumor Immunostaining for the Pan-B-Cell Antigen (CD20) and the EBV Receptor (CD21).

SOURCE OF SPECIMEN	TUMOR TYPE	DEGREE OF REACTIVITY	
		CD20	CD21
HIV-positive patients			
1	Leiomyosarcoma	None	Strong
	Leiomyoma	None	Strong
2	Leiomyosarcoma	None	Strong
3	Leiomyosarcoma	None	Strong
4	Leiomyosarcoma	None	Weak
5	Leiomyosarcoma	None	Strong
6	Leiomyoma	None	Strong
HIV-negative patients			
7	Leiomyosarcoma	None	Weak
8	Leiomyosarcoma	None	Weak
9	Leiomyosarcoma	None	Weak
10	Leiomyoma	None	Weak
11	Leiomyoma	None	Weak
12	Leiomyoma	None	Weak
13	Leiomyoma	None	Weak
Normal tissues			
Tonsil epithelium	—	Strong	Strong
Smooth muscle	—	None	Weak
Striated muscle	—	None	Weak

transformation of myocytes. The presence of EBV in both a leiomyoma and leiomyosarcoma in one patient (Patient 1) suggests that EBV infection precedes malignant transformation. Another important indication that EBV infected the muscle cells before their trans-

formation is the monoclonality of the EBV in two tumor specimens from Patient 5, obtained at different times from different sites. The most plausible explanation of this result is that the tumors arose from two different EBV-infected myocytes (Fig. 2). The bicolon EBV in the tumor from Patient 4 is consistent with either a simultaneous infection of tumor cells by two different EBV viruses (an unlikely possibility) or a tumor-cell population derived from two EBV-infected precursor cells (a likely occurrence). The nearly identical intensity of the two bands representing the terminal fragments of EBV suggests that the extent of viral proliferation was similar in each clone. The lack of EBV polyclonality in these specimens argues against infection of the myocytes after they had been transformed into leiomyosarcomas. The finding of molecularly distinct viruses in two tumors from one of these patients (Patient 5) suggests that EBV infection of smooth-muscle cells is not uncommon in young people with AIDS. Decreased immune surveillance, increased expression of the EBV receptor, and high levels of EBV in plasma may all contribute to the pathogenesis of smooth-muscle tumors in such patients.

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Figure 3. Southern Blot Hybridization with the *EcoRI I* and *XhoI a* Probes from the Two Ends of the Linear EBV Genome.

The identical findings with the two probes indicate that there are only episomal forms of the virus (solid triangles) and no integration of EBV DNA into the host-cell genome. DNA from the tumor in Patient 4 (10 μ g) shows two distinct bands with both probes, revealing the presence of two clones of EBV within the tumor. DNA from the two tumors (lanes A and B) in Patient 5 (10 and 1.5 μ g, respectively) shows two different bands with the *XhoI a* probe. Thus, these two tumors are monoclonal but infected with different EBV clones. The sample in lane A contained insufficient DNA for the detection of EBV by the *EcoRI I* probe. The expected EBV monoclonal episomal forms were found in Raji and B95-8 cells (20 and 2 μ g, respectively), in addition to the linear, replicative forms of EBV in B95-8 cells (open triangles).

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