

THE ASSOCIATION OF EPSTEIN-BARR VIRUS WITH SMOOTH-MUSCLE TUMORS OCCURRING AFTER ORGAN TRANSPLANTATION

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Abstract Background. Epstein-Barr virus (EBV) has been associated with nasopharyngeal carcinoma, some lymphomas, and lymphoproliferative disease after organ transplantation. Many lymphoproliferative tumors that occur after transplantation are clonal, a property that classifies them as neoplastic. Clonality can be determined by analysis of the extrachromosomal circular DNA episomes produced by EBV infection.

Methods. We describe three young children in whom smooth-muscle tumors developed 18 months to 5½ years after liver transplantation with immunosuppression. We examined the tumors by microscopy and with immunohistochemical studies and molecular genetic analyses of the EBV DNA.

Results. The tumors were composed of spindle cells with smooth-muscle features and resembled those described in patients with the acquired immunodeficiency syndrome. Immunohistochemical analysis was negative for EBV latent membrane protein and EBV receptor (CD21), but positive for EBV nuclear antigen 2. In situ hybridization revealed nuclear EBV sequences, and molecular genetic analysis showed the EBV genome to be clonal in all three patients.

Conclusions. Smooth-muscle tumors that developed after organ transplantation contained clonal EBV, suggesting that the virus has a role in the development of these neoplastic lesions. (N Engl J Med 1995;332:19-25.)

IMMUNODEFICIENT patients have an increased incidence of neoplasms, whether the immunodeficiency is due to genetic disorders, the acquired immunodeficiency syndrome (AIDS), or immunosuppressive therapy.¹ A lymphoproliferative disorder sometimes indistinguishable from lymphoma occurs in patients with AIDS and the recipients of organ transplants. Clonal Epstein-Barr virus (EBV) genome has been found in many lymphoproliferative tumors²⁻⁴ and may be causative.⁵ Other neoplasms have been described in patients with AIDS,¹ including smooth-muscle tumors.⁶⁻¹¹

We describe three children who underwent organ transplantation in whom smooth-muscle tumors developed during immunosuppression. These tumors were all clonal, containing forms of EBV DNA that directly link their neoplastic pathogenesis to the virus:

CASE REPORTS

Patient 1

Patient 1 has been described previously.¹² She underwent a Kasai procedure for extrahepatic biliary atresia at the age of 2 months and received a sex-mismatched liver transplant at the age of 18 months. Three years later a mass 3 cm in diameter and two satellite nodules composed of smooth-muscle cells were resected from the left lobe of the liver. The child is now 7½ years old; there has been no recurrence, and she is negative for the human immunodeficiency virus (HIV).

Patient 2

Patient 2 underwent a Kasai procedure for extrahepatic biliary atresia at the age of 1 month and received a sex-matched liver trans-

plant at the age of 15 months. Cyclosporine, corticosteroids (methylprednisolone and prednisone), and azathioprine were given for immunosuppression; a course of muromonab-CD3 (Orthoclone OKT3, Ortho) was administered during the first month after transplantation. Five and a half years after transplantation, when the child was 6¾ years old, intermittent sharp abdominal pain, decreased appetite, weight loss, and fever developed. Bilateral anterior cervical lymphadenopathy prompted an evaluation for lymphoproliferative disease. Serologic tests for EBV indicated an active infection. The results of serologic analysis for HIV were negative. Computed tomographic scans revealed multiple small nodules in the lung and liver and a retroperitoneal mass 10 cm in diameter. Endoscopy revealed multiple nodules in the stomach and colon. The nodules in the left main bronchus, liver, stomach, and colon were smooth-muscle lesions; no lymphoproliferative disease was found. Immunostaining for EBV latent membrane protein was negative. The intensity of immunosuppressive therapy was reduced, and intravenous acyclovir was initiated. The aggressive tumor behavior was treated by chemotherapy (vincristine sulfate, dactinomycin, and cyclophosphamide), with no change in the size of the tumor. Candidal infection of the gastrointestinal tract, candidemia, and multiorgan failure developed, and the patient was removed from life support and died. At autopsy, tumors were found in the heart, lungs, liver, stomach, small bowel, colon, and retroperitoneum. *Candida albicans* was found at numerous sites.

Patient 3

Patient 3 had intestinal volvulus at two days of age requiring extensive resection of the small bowel. Liver failure developed while she was receiving total parenteral nutrition. The results of serologic analysis were consistent with a past infection with EBV. At 20 months of age she underwent transplantation of the liver and small bowel during immunosuppression with tacrolimus (FK 506, Fujisawa, Deerfield, Ill.). Corticosteroids and azathioprine, but not muromonab-CD3, were given to prevent rejection. Eight months after transplantation, lymphoproliferative disease involving the anterior mediastinal and paravertebral lymph nodes, lung, and stomach was found. Immunosuppressive therapy was stopped, with regression of the lymphoproliferative disease, and was later reinstated because of rejection. Twelve months after transplantation, smooth-muscle tumors were found in native colon. The results of serologic analysis were consistent with a resolving EBV infection. The colonic nodules shrank with a reduction in the dose of immunosuppressive therapy, but the rapid onset of rejection necessitated resumption of the earlier level of immunosuppression. The results of serologic analysis for HIV were negative. The patient's condition deteriorated with persistence of the tumor, and she died at the age of three years. No autopsy was performed.

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METHODS

Light Microscopy

Tissue was fixed in neutral buffered formalin, post-fixed in alcohol–zinc formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, Ziehl–Neelsen, Fite, Masson's trichrome, and periodic acid–Schiff with diastase. The tumor specimens were evaluated with respect to cellular morphology, cellularity, necrosis, nuclear pleomorphism, and the mitotic count. The numbers of mitotic figures were counted in five sets of 10 high-power fields (approximately 9 high-power fields = 1 mm²). The highest number of mitotic figures in one set of 10 high-power fields constituted the final mitotic count.

Electron Microscopy

Tissues from Patients 1 and 2 were fixed in a solution of 4 percent paraformaldehyde and 0.5 percent glutaraldehyde, post-fixed in 1 percent osmium tetroxide, and embedded in Epon–Araldite. Sections cut on an ultramicrotome (Nova, LKB, Rockville, Md.) were stained with 2 percent uranyl acetate and 0.3 percent lead acetate and examined and photographed with an electron microscope (CM-10, Phillips, Mahwah, N.J.).

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded sections of tissue from all three patients were deparaffinized and rehydrated. Protease digestion was used for factor VIII–related antigen. The slides were immunostained with the avidin–biotin–peroxidase method (Vector Laboratories, Burlingame, Calif.), in which 3,3'-diaminobenzidine was used as the chromogen, and counterstained with Gill's hematoxylin. The following antibodies were used: muscle-specific actin (Enzo Biochem, New York), desmin (Bio Genex, San Ramon, Calif.), factor VIII–related antigen (Dako, Carpinteria, Calif.), S-100 protein (Dako), vimentin (Bio Genex), EBV latent membrane protein (Dako), KP-1 (CD68) (provided by D. Mason, Oxford, United Kingdom), factor XIIIa (Calbiochem, La Jolla, Calif.), EBV nuclear antigen 2 (EBNA-2) (Novocastra Laboratories, Newcastle-upon-Tyne, United Kingdom), CD21 (Dako), biotinylated antimouse IgG (Vector), and biotinylated anti-rabbit IgG (Vector).

Immunostaining for CD21 and EBNA-2

Tissue sections frozen in Optimal-Cutting-Temperature compound (Miles Laboratories, Elkhart, Ind.) from Patients 1 and 2 were fixed in cold acetone, air dried, depleted of endogenous peroxidase activity, and immunostained for CD21 and EBNA-2 as above with 3-amino-9-ethylcarbazole (Biomedica, Foster City, Calif.) as the chromogen.

In Situ Hybridization for *EBER*

Formalin-fixed, paraffin-embedded sections of tumor from all three patients were mounted on slides treated with 3-aminopropyltriethoxysilane (Probe-On, Fisher Scientific, Pittsburgh), deparaffinized, digested with pronase, and dehydrated. The EBV *EBER-1* (*EBER*) probe, a probe of 30 base pairs that recognizes a region of the EBV genome transcribed in latently infected cells, was applied. The slides were incubated at 95°C for 10 minutes and 37°C overnight before being washed with graded saturated solutions of sodium citrate and treated with streptavidin and peroxidase (Boehringer–Mannheim, Indianapolis). The color was brought out by 3-amino-9-ethylcarbazole and counterstaining with hematoxylin.

Immunohistochemical analysis for muscle-specific actin and in situ hybridization for *EBER* was performed in tandem on specimens from Patient 3.

In Situ Hybridization for the Y Chromosome

Formalin-fixed, paraffin-embedded sections of tumor and liver allograft from Patient 1 (who received a sex-mismatched transplant) were mounted on glass slides coated with 3-aminopropyltriethoxysi-

lane, deparaffinized, digested with pepsin, and dehydrated. A biotinylated 3.4-kb DNA probe specific for the Y chromosome, which recognizes a repeat sequence in Y chromosome heterochromatin of humans,¹³ was applied. The slides were incubated at 95°C for 15 minutes and 42°C overnight before being washed with formamide and graded saturated solutions of sodium citrate. Sections equilibrated in phosphate-buffered saline were incubated in substrate at room temperature for 30 minutes. The color was brought out by diaminobenzidine. The slides were stained with eosin, rehydrated, stained with methyl green, dehydrated, and cleared in xylene, and coverslips were laid over them. As a control, tonsillar tissue from both girls and boys was tested in parallel.

Immunohistochemical Analysis for HLA Antigens

Sections of tissue from Patient 2 that had been frozen in Optimal-Cutting-Temperature compound were mounted on slides treated with silane, fixed in cold acetone, placed in a humid chamber, and incubated with blocking agents — avidin and biotin (Vector), protein-blocking agent (Shandon-Lipshaw-Immunon, Pittsburgh), and normal serum (Vector). Mouse antihuman monoclonal antibodies directed against donor-specific and recipient-specific HLA antigens, as determined by HLA typing by the complement-dependent lymphocytotoxicity test (the standard method used by the National Institutes of Health), were applied, and the slides were incubated with HLA antigens and secondary antibodies, avidin–biotin–peroxidase solution, 3-amino-9-ethylcarbazole, and hematoxylin.

Cytogenetic Analysis

Tumor cells from the retroperitoneal mass obtained at autopsy from Patient 2 were cultured and banded according to Klinger's method,¹⁴ with analysis of 20 cells in metaphase that had been partially digested with trypsin and stained with Giemsa stain. Tissue from Patient 1 was not available for analysis; the tumor cells from Patient 3 had bacterial overgrowth.

Purification, hybridization, and Southern blot analysis of DNA were performed as previously described.⁴

RESULTS

Light Microscopy

On light microscopy, all tumors exhibited interlacing fascicles of spindle cells with eosinophilic cytoplasm and elongated, blunt-ended nuclei with finely stippled chromatin (Fig. 1A). Focal mild pleomorphism and small areas of necrosis were seen. There was less than 1 mitotic figure per 1.1 mm². No atypical lymphoid cells characteristic of EBV infection were seen in tumor tissue or adjacent normal tissue.

Electron Microscopy

The liver tumor from Patient 1 and the lung and liver tumors from Patient 2, examined on electron microscopy, were composed of smooth-muscle cells with cytoplasmic thin filaments, cytoplasmic electron-dense bands, electron-dense plaques along plasma membranes, basement membranes, and pinocytotic vesicles (Fig. 1B). There were occasional intermixed myofibroblasts. No specimens from Patient 3 were available for electron microscopy.

Immunohistochemical Analysis

Tumor cytoplasm showed immunostaining for vimentin and muscle-specific actin and focal immunostaining for desmin (Table 1). No staining for S-100

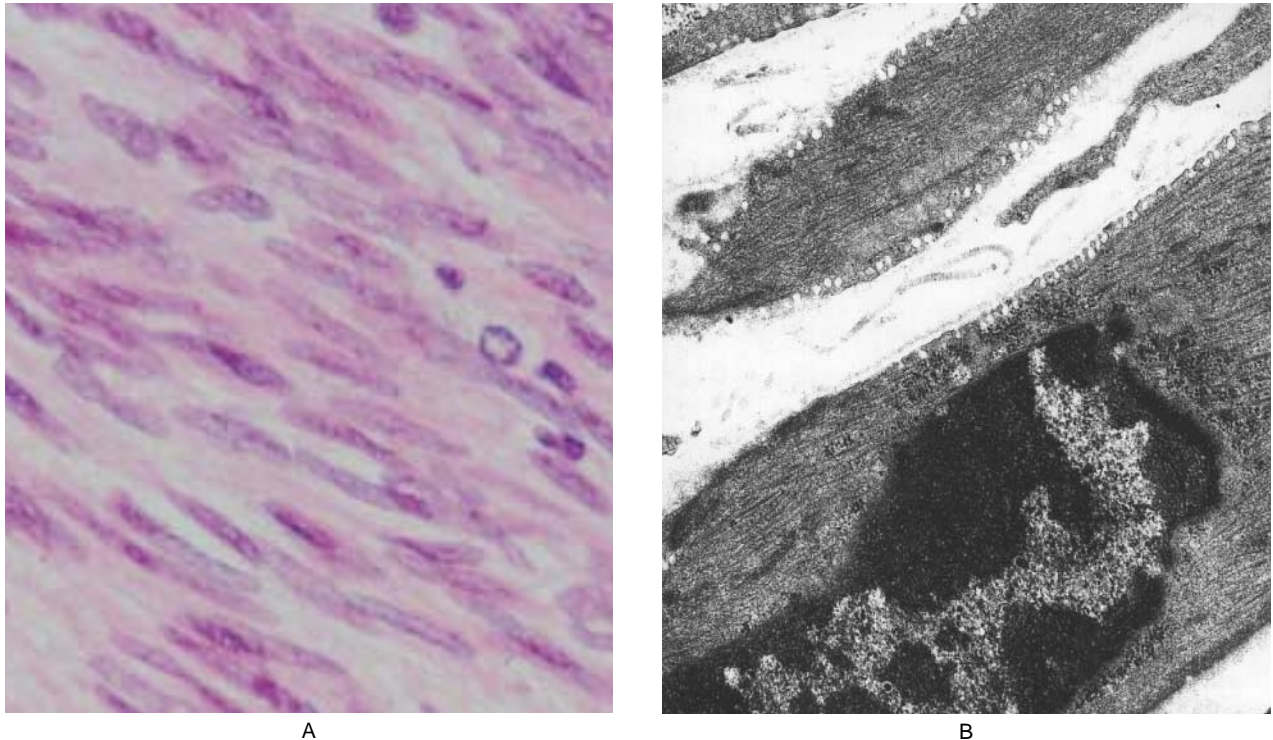


Figure 1. Light (Panel A) and Electron (Panel B) Photomicrographs of Tumor Tissue from the Liver Allograft of Patient 1.

As can be seen in Panel A, the tumor is composed of fascicles of spindle cells with elongated nuclei. No mitotic figures are present (hematoxylin and eosin, $\times 128$). The findings in Patients 2 and 3 were similar. As can be seen in Panel B, the tumor had features characteristic of smooth muscle, including cytoplasmic thin filaments, electron-dense bands and plaques, basement membrane, and pinocytotic vesicles ($\times 6300$). The findings in tumor tissue from Patient 2 were similar. Panel B is reprinted from Doyle et al.¹² with the permission of the publisher.

protein or factor VIII-related antigen was observed. CD68 and factor XIIIa were found in interstitial cells but not smooth-muscle cells. All tumor and nontumor cells were negative for EBV latent membrane protein, with the exception of cells from the retroperitoneal tumor from Patient 2, which had a few positive cells.

Immunostaining for EBNA-2 was positive in at least 50 percent of tumor cells from Patients 1 and 2 (Fig. 2), but negative in parenchymal and stromal cells. Staining for CD21 was negative in Patients 1 and 2. Immunostaining for EBNA-2 and CD21 was not performed in Patient 3 because of a lack of tissue.

Immunohistochemical analyses for HLA antigens were limited by the availability of antibodies specific for donor HLA that had no cross-reactivity or specificity for the HLA of Patient 2. Only one antibody directed against donor HLA was available. A minimum of two antibodies directed against donor HLA and one antibody directed against recipient HLA are required, making our findings inconclusive. No tissue from Patient 3 was available for study.

In Situ Hybridization

In situ hybridization of hepatocytes and tumor cells in liver tissue from Patient 1 revealed deeply staining intranuclear dots in most cells (not shown), indicating

the presence of the Y chromosome. Lymphoid cells were negative for the Y chromosome.

With respect to in situ hybridization for *EBER*, the nuclei of most tumor cells from each patient were strongly positive (Fig. 3), whereas the nuclei in adjacent parenchymal and stromal cells were negative for *EBER*. In Patient 2 there were rare positive hepatocytes and stromal cells far from the neoplasm.¹⁵

The combination of immunohistochemical analysis and in situ hybridization of tumor cells from Patient 3 produced evidence of antibody against muscle-specific actin in the cytoplasm and of EBV nucleic acid (Fig. 3).

Cytogenetic Analysis

The karyotype of retroperitoneal-tumor tissue from Patient 2 was 46,XX. One cell had a karyotype of 45,XX,-15, one had a karyotype of 44,XX,-7,-18, and one had a karyotype of 44,X,-X,-2,+mar. Many cells had prominent satellites on one chromosome 21 (normal polymorphic variant). No consistent numerical or structural abnormalities were observed.

Molecular Genetic Analysis

Clonal EBV DNA was detected in all tumor tissue studied (Fig. 4). In Patient 1 a clonal band present at a frequency of about 25 copies per haploid genome dom-

Table 1. Results of Immunohistochemical Analysis of Tumor Specimens from Three Patients with Smooth-Muscle Tumors after Transplantation.*

PATIENT NO.	VIMENTIN	MUSCLE-SPECIFIC	DESMIN	S-100	FACTOR VIII-	EBV LATENT	EBNA-2	CD21
		ACTIN		PROTEIN	RELATED ANTIGEN	MEMBRANE PROTEIN		
<i>percentage of cells stained</i>								
Patient 1								
Liver tumor	≥50	≥50	1-9	0	0	0	≥50	0
Patient 2								
Tumor of left main bronchus	≥50	≥50	0	0	0	0	≥50	0
Gastric tumor	≥50	≥50	0	0	0	0	ND	ND
Retroperitoneal tumor	1-9	≥50	0	0	0	0†	ND	ND
Liver tumor	≥50	≥50	1-9	0	0	0	≥50	0
Patient 3								
Colonic tumor	10-49	≥50	1-9	0	0	0	ND	ND

*ND denotes not done.

†There were a few positive cells.

inated the hybridization pattern; the presence of other, much weaker bands suggested minor episomal subpopulations. A low-molecular-weight band representing linear virion DNA was also detected, suggesting lytic EBV infection. In retroperitoneal-tumor tissue from Patient 2, a single clonal EBV band was present at a frequency of about five copies per haploid genome. The lung-tumor specimen from Patient 2 showed two weaker bands, each present at a frequency of approximately one copy per haploid genome. In Patient 3, a single clonal band was present at a frequency of about three copies per haploid genome.

Specimens from the two tumors studied in Patient 2 had different patterns of EBV DNA hybridization, but both had a common polymorphism at a *Bgl*II restriction site, demonstrating that they contained the same strain of EBV, most likely from the initial viral infection.

The lung-tumor specimen from Patient 2 had two high-molecular-weight bands of equal strength, each present as a single copy. Mapping studies indicated that the pattern represented chromosomal integration at the linear ends, an unusual event for EBV.¹⁶

No evidence of rearrangements of immunoglobulin genes or T-cell-receptor genes was found in any tumor.

DISCUSSION

Analysis of EBV DNA in the smooth-muscle tumors from our three patients revealed features similar to those found in lymphoproliferative disease after transplantation,^{3,4} including unique EBV DNA episomes, whose presence suggests two important features. First, the single form of EBV DNA present in each tumor indicates that it was the abnormal outgrowth of a single clone of cells, a defining feature of neoplasia. Second, the presence of unique episomal bands suggests that EBV was present before the clonal population was derived. EBV infection of established tumors would have produced polyclonal patterns.

As in most clonal forms of lymphoproliferative dis-

ease that occur after transplantation, tumor tissue from Patient 1 contained detectable levels of other forms of EBV DNA,⁴ although the levels of these forms were far lower than the levels of the main episome. These forms may have occurred for several reasons. The clonal proliferation may have arisen from a nonclonal viral infection. The minor species could be remnants of the original infection and thus a clue to neoplastic progression. The recombination and deletion of episomes, especially at high copy numbers, will produce forms of various sizes. As in post-transplantation lymphoproliferative dis-

ease, occasional cells may reactivate lytic virus infection, giving rise to virions that can reinfect tumor cells and infect stromal infiltrating lymphocytes.

The chromosomal integration of EBV DNA in Patient 2 is of special interest because it is rarely observed. The presence of polymorphisms indicates that the two tumors were derived from the same EBV infection, but they might still be independent tumors. It

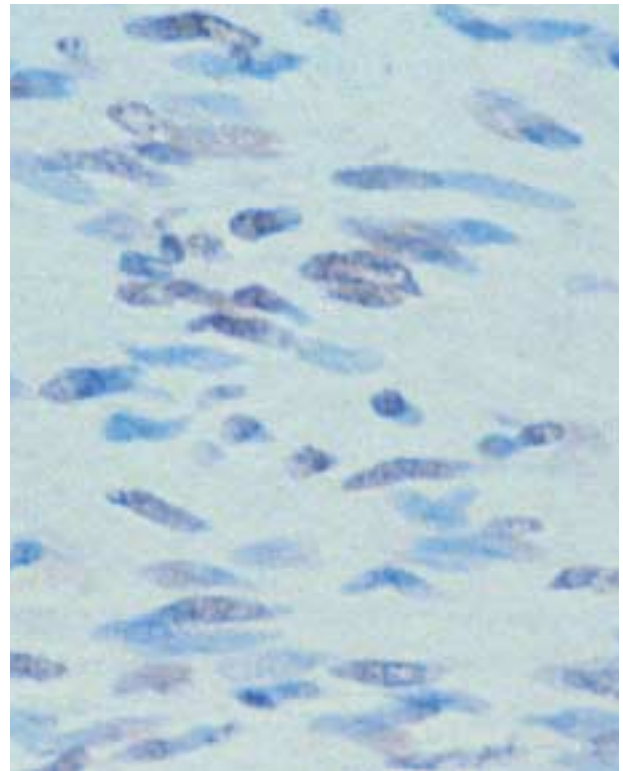


Figure 2. Immunostaining for EBNA-2 in Retroperitoneal-Tumor Tissue from Patient 2 (×128).

Many nuclei are stained reddish brown, indicating a positive result. The results for Patients 1 and 3 were similar.

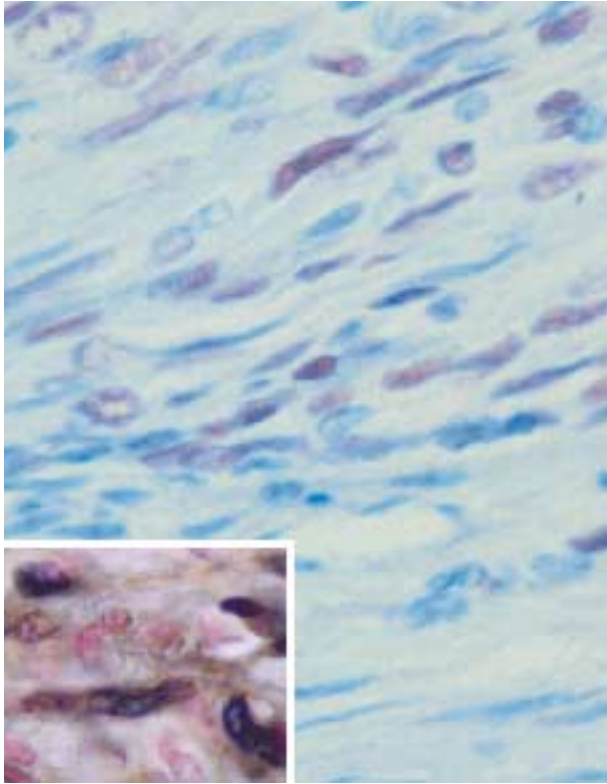


Figure 3. In Situ Hybridization with the *EBER* Probe of a Lung-Tumor Specimen from Patient 2 ($\times 128$).

There is strong, uniformly positive staining of nuclei in tumor cells (reddish-brown areas) but no staining of nuclei in compressed normal stroma (lower right). The inset shows the results of immunohistochemical analysis of tissue from a native-colon tumor in Patient 3 with the use of antibody against muscle-specific actin, followed by in situ hybridization with the *EBER* probe. The tumor cells are positive for both actin (brown areas) and *EBER* RNA (blue-black areas) ($\times 400$).

is likely, in view of the anatomical findings, that the lung tumor metastasized from the retroperitoneal tumor. The differences between the two tumors may be explained as follows. The original clonal cell population contained a circular EBV DNA episome, but re-emergence of lytic gene expression produced linear DNA molecules. A linear DNA molecule was integrated into chromosomal DNA. The episomal DNA was lost in a daughter cell; episomes are easily lost during cell division, since they are not segregated as chromosomes are. If, however, expression of the EBV gene was essential for tumor progression, daughter cells would be lost as the tumor progressed unless they retained EBV DNA in another form (i.e., through integration). A subclone derived from a daughter cell then gave rise to the metastasis.

The clinical behavior of the smooth-muscle tumors varied. Patient 1 presented without symptoms, and the liver tumor did not recur after resection. In Patient 2, the tumors resulted in abdominal pain, decreased ap-

petite, weight loss, fever, and obstruction of the left lower lobe with atelectasis and pneumonia. In Patient 3, the tumors were asymptomatic and the diagnosis was made incidentally on endoscopy. Patients 2 and 3 had no response to antiviral or antineoplastic therapy. Since acyclovir inhibits viral replication, antiviral therapy would be expected to have little effect on the tumor cells, which had latent EBV infection.⁵ The effect of modulating immunosuppressive therapy was also difficult to assess in these patients. In Patient 3, tumor shrinkage followed a reduction in the dose of immunosuppressive therapy, but the rapid onset of rejection necessitated the reinstatement of the former dose of immunosuppression. Only Patient 2 received chemotherapy, without apparent effect; she died of infection after three cycles.

The histologic features of these tumors were not predictive of biologic behavior. All tumors had less than 1 mitotic figure per 1.1 mm², moderate cellularity, fine chromatin, inconspicuous nucleoli, and focal mild pleomorphism. According to published criteria¹⁷ the tumors were benign or of uncertain malignant potential, yet the lesions in Patient 2 behaved aggressively, progressing even during chemotherapy.

Smooth-muscle tumors have been reported in children and an adult with HIV.⁶⁻¹¹ The tumors were multifocal, with various distributions and presentations, similar to the tumors that appeared after transplantation. Five children with AIDS had liver tumors that were resected without recurrence or were incidental findings on autopsy. The patients died of infection, cachexia, or HIV encephalopathy.¹¹ Other smooth-muscle tumors in patients with AIDS occurred in the lung, colon, and ileum.

Serologic analysis for EBV in patients with AIDS and smooth-muscle tumors has been limited to one patient who had elevated titers of EBV nuclear antigen.¹¹ In situ hybridization for EBV was negative in a hepatic-tumor specimen from one child with AIDS.⁶ In another case,¹¹ in situ hybridization demonstrated EBV RNA in tumor-cell nuclei, similar to our findings, suggesting that for both transplant recipients and those with AIDS the development of smooth-muscle tumors is related to EBV infection.

EBV-related neoplasms have one of two patterns of gene expression directed through distinct viral promoters.¹⁸ In one pattern, tumors such as Burkitt's lymphoma express only EBV nuclear antigen 1, which does not appear to be a target for cytotoxic lymphocytes. In contrast, lymphoproliferative tumors that occur after transplantation express a different variety of highly immunogenic proteins, including EBNA-2, suggesting that tumors that are positive for EBNA-2, such as the smooth-muscle tumors, preferentially arise in the presence of immunosuppression.

The expression of EBNA-2 is associated with early latent EBV infection and is required for the immortalization (indefinite growth) of B lymphocytes in vitro.

Activities induced by EBNA-2 include an increase in the expression of viral or cellular RNA or protein.¹⁹ Immunostaining of the smooth-muscle tumors for CD21, which is nearly identical to the B-lymphocyte and epithelial EBV receptors,²⁰ was negative. EBV latent membrane protein, which has transforming properties, is probably required for the immortalization of B lymphocytes¹⁹ and is generally expressed with EBNA-2¹⁸; however, immunostaining for latent membrane protein was largely negative. The negative results do not exclude the possibility that EBV receptors or

EBV latent membrane protein is expressed by tumor cells; the level of such expression may be below the threshold of sensitivity of the technique, or the antibodies may not recognize antigens on smooth-muscle cells.²¹ Various patterns of expression of EBNA-2 and latent membrane protein have been observed in latent EBV infection, including that causing lymphoproliferative disease, nasopharyngeal carcinoma, and Burkitt's lymphoma.^{18,22-24} The lack of expression of latent membrane protein is not unique and does not exclude the possibility of latent EBV infection. Restricted expression of EBV latent genes and associated down-regulation of cellular adhesion molecules may help the infection go undetected by the T-cell immune-surveillance system and thus offer a selective advantage.² Proliferations of spindle cells representing exuberant histiocytic inflammatory responses associated with mycobacterial infection have been described in patients with AIDS,^{25,26} but no features of smooth-muscle cells were identified in those lesions.

The findings of a clonal EBV genome and the expression of *EBER* and EBNA-2 strongly support a causative role for EBV in the development of smooth-muscle tumors after transplantation. The exact nature of

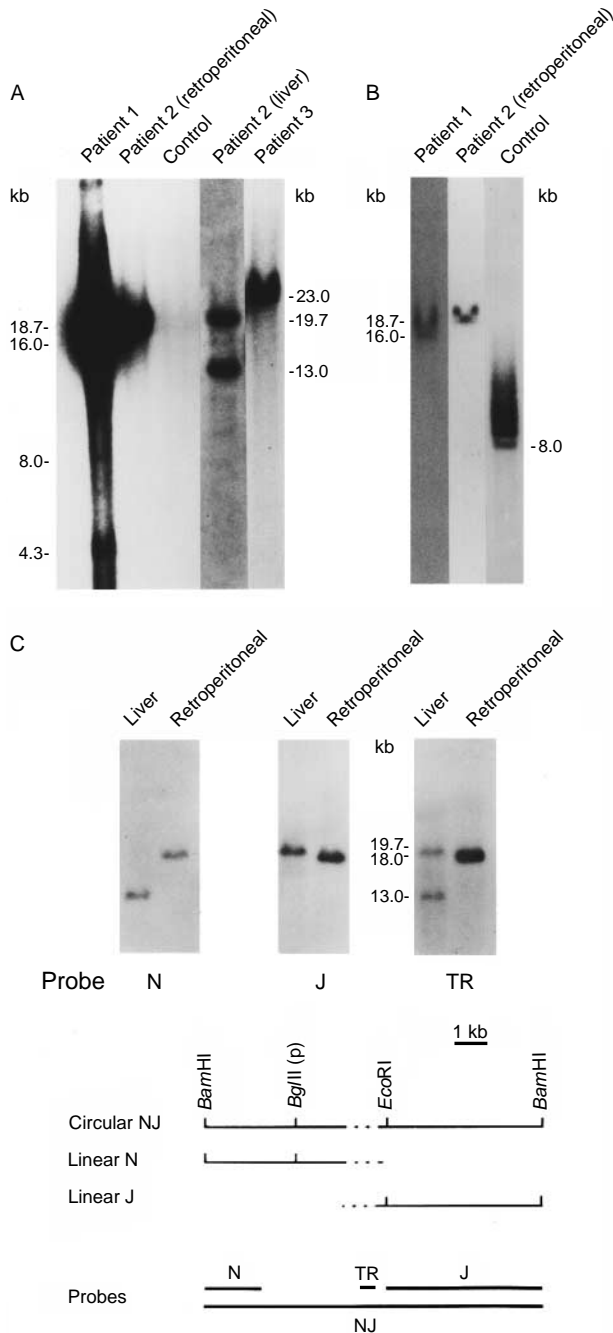


Figure 4. Southern Blots of EBV DNA in Tissue from Patients 1 and 2.

Panels A and B show the results of hybridization with the NJ probe. The use of normal exposure times for autoradiography resulted in a relative abundance of EBV genomes in four tumor specimens and in control DNA from a normal lymph node (Panel A). The two bands in the liver-tumor specimens from Patient 2 were each present as a single copy. The 4.3-kb band near the bottom of the lane showing the results for Patient 1 is characteristic of linear virion DNA, suggesting a detectable level of lytic virus infection. The use of reduced exposure times revealed the clonal patterns (Panel B). The tissue samples from Patients 1 and 2 contained circular EBV DNA of a single size, indicating a clonal cell population. In contrast, the control sample shows the polyclonal EBV DNA pattern characteristic of mononucleosis, with a regular pattern of bands representing circular DNA species of 8.0 kb, 8.5 kb, 9.0 kb, and so on.

Panel C shows the results of studies to characterize the integration of EBV DNA into the liver and retroperitoneal tumors in Patient 2. DNA was digested with *Bam*HI and hybridized with three probes from different regions of the EBV DNA termini. DNA concentrations were adjusted to improve resolution and normalize band intensity. In the retroperitoneal tumor, all three probes hybridized to the same band, demonstrating an intact circular (episomal) DNA with fused termini. In the liver tumor, since one band contained the left end of linear virion DNA and the other the right end, the ends were unlinked. The bands were much larger than those of linear virion DNA, indicating linkage to chromosomal DNA at the linear ends. Additional studies showed that tissue from both the liver tumor and the retroperitoneal tumor contained the same *Bgl*II polymorphism (p), indicating that both tumors were derived from a common strain of EBV, presumably from the initial viral infection. The maps at the bottom show the probes and restriction enzymes used to analyze the EBV termini. N and J are terminal segments of linear EBV DNA, which join on circularization to form the fused NJ segment. TR denotes a 0.5-kb terminal-repeat sequence present in variable copy numbers at the ends or in fused NJ segments.

the interaction between EBV, immunosuppression, and tumor formation in this setting remains to be established.

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