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A New Primary Effusion Lymphoma-Derived Cell Line Yields a Highly Infectious Kaposi's Sarcoma Herpesvirus-Containing Supernatant

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A primary effusion lymphoma (PEL) cell line, JSC-1, that yields highly infectious Kaposi's sarcoma herpesvirus (KSHV) supernatants was established from the ascitic fluid of a human immunodeficiency virus-positive patient. Flow cytometry showed strong expression of CD45 and lambda light-chain restriction. Southern blot hybridization showed immunoglobulin heavy-chain gene rearrangements in the tumor and the resultant cell line consistent with B-cell lineage. Expression of viral genes was assessed by reverse transcription-PCR and immunohistochemistry. Only latent Epstein-Barr virus (EBV) gene expression was detected, and this was at a low level. In contrast, lytic and latent KSHV gene expression were detected. Tetradecanoyl phorbol acetate and butyrate upregulated KSHV lytic expression, but not EBV lytic expression. Viral supernatant from JSC-1 was much more efficient at infecting primary human dermal microvascular endothelial cells (DMVECs) with KSHV than supernatants from BC-3 or BCP-1 PEL cell lines. Quantitation of viral yields produced by the PEL lines showed at least 2 orders of magnitude more DNase I-resistant KSHV DNA in the JSC-1 supernatant compared to BC-3 or BCP-1 supernatants. KSHV infection in DMVECs was associated with a change from a cobblestone to a spindle shape, LANA expression, and an increased number of mitoses.

The Kaposi's sarcoma herpesvirus (KSHV), or human herpesvirus 8 (HHV-8), was first discovered in association with Kaposi's sarcoma lesions in AIDS patients (8). Since then, the virus has been consistently detected in all epidemiologic forms of Kaposi's sarcoma as well as in primary effusion lymphoma (PEL) and a subset of Castleman's disease (4–6, 11, 29). Sequence analysis, expression of cloned viral genes from recombinant vectors in model systems, immunohistochemistry, in situ hybridization, PCR, and reverse transcription-PCR (RT-PCR) of infected tissues have provided tantalizing glimpses of the role that KSHV may play in the pathogenesis of disease. However, understanding has been limited by the lack of availability of a tractable system for studying primary infection in vitro.

In the attempt to develop an in vitro infection model, several sources of virus have been used, including primary Kaposi's sarcoma specimens and supernatants of PEL-derived cell line cultures (12, 13, 20, 22, 24). In this report, we characterize a new PEL cell line, JSC-1, that shows higher basal and induced expression of KSHV lytic cycle gene products (viral interleukin-6 [vIL-6], T1.1/nut1, and viral thymidine kinase [vTK]) than do the BC-3, BCBL-1, and HBL-6 cell lines. JSC-1 yields supernatant virions that are highly infectious in a new in vitro infection assay using primary endothelial cell cultures (D. M. Ciufo, J. S. Cannon, L. J. Poole, F. Wu, P. Murray, R. F. Ambinder, and G. S. Hayward, unpublished data).

MATERIALS AND METHODS

Cell line establishment and culture. Mononuclear cells were isolated from ascites fluid by Ficoll-Hypaque (Pharmacia Biotech AB, Uppsala, Sweden) density gradient centrifugation followed by three washes in sterile phosphate-buffered saline. Cells were seeded at 5×10^5 cells/ml in RPMI 1640 supplemented with 10% fetal bovine serum, 50 μg of gentamicin/ml, and 2 mM L-glutamine and incubated at 37°C in 5% CO_2.

Other cell lines used in this report include the Epstein-Barr virus-positive [EBV(+)]/KSHV(+) PEL cell lines HBL-6 and BC-2 (7, 14); the EBV(−)/KSHV(+) PEL cell lines BCBL-1, BC-3, and BCP-1 (1, 3, 25); the EBV(+) marmoset cell line B95-8; an EBV(+) human lymphoblastoid cell line (LCL) transformed with B95-8 supernatant; and the EBV(−) Burkitt's lymphoma cell line CA46. Cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum (HBL-6, JSC-1, BCBL-1, B95-8, LCL, and CA46) or with 20% fetal bovine serum (BC-2, BC-3, and BCP-1). Primary adult dermal endothelial cells (HMVEC-d Ad, Clonetics catalog no. CC 2543) were grown in Clonetics EGM2-MV Bullet-kit medium (catalog no. CC-3202). Cells from up to passage 6 were used.

Immunophenotypic analysis. The immunophenotypes of the JSC-1 tumor cells and the derivative cell line were determined by flow cytometry (2). Cells were stained with a panel of monoclonal antibodies conjugated to fluorescein isothiocyanate, phycoerythrin, or peridin chlorophyll alpha protein and were analyzed by flow cytometry on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). A minimum of 5,000 events was collected for each antibody combination. Data were analyzed using Paint-a-Gate Pro software (Becton Dickinson).

Nucleic acid extraction. Genomic DNA was extracted from cells by digestion with proteinase K, extraction with phenol-chloroform, and ethanol precipitation (26). Total RNA was isolated from the JSC-1 cell line after 6 months of passage using the TRIzol reagent (Gibco BRL, Gaithersburg, Md.), according to the manufacturer's instructions.

RT and PCR amplification. RT-PCR used 1 μg of RNA and the Gene Amp RNA PCR kit (Perkin-Elmer, Foster City, Calif.) according to the manufacturer's instructions. The 20-μl reaction mixture was incubated for 45 min at 42°C in the presence of oligo$(dT)_{16}$ primers. PCR primers and internal probes for the EBV transcripts have been previously described (32). PCR amplifications involved initial denaturation at 95°C for 3 min, followed by 39 cycles consisting of 94°C for 30 s, optimal annealing temperature for 1 min, and 72°C for 1 min, and then a final extension at 72°C for 10 min. The RT-PCR products were electrophoresed on a 1.8% agarose gel, transferred to a Hybond N^+ membrane, and hybridized with a ^{32}P end-labeled internal oligonucleotide probe. Hybridizations using the Rapid-Hyb buffer system (Amersham, Arlington Heights, Ill.) were at 52°C for 2 h. The membrane was washed and exposed at −80°C with intensifying screens for 1 h or overnight. PCR analysis was used to determine strain-specific sequence variation within the EBNA-3C coding region (27). Oligonucleotide primers complementary to EBV types 1 and 2 but spanning a type-specific deletion were as follows: 5′-AGAAGGGGAGCGTGTGTTGT-3′ and 5′-GGC TCGTTTTTGACGTCGGC-3′.

Chemical induction. Fresh stocks of PEL cell lines were used for induction experiments. Cells were diluted to 3×10^5 cells/ml with fresh media. On the following day, chemical inducers were added and cells were incubated with

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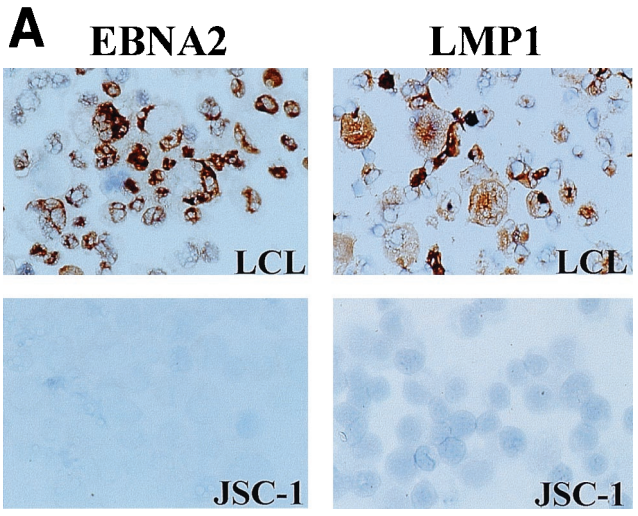


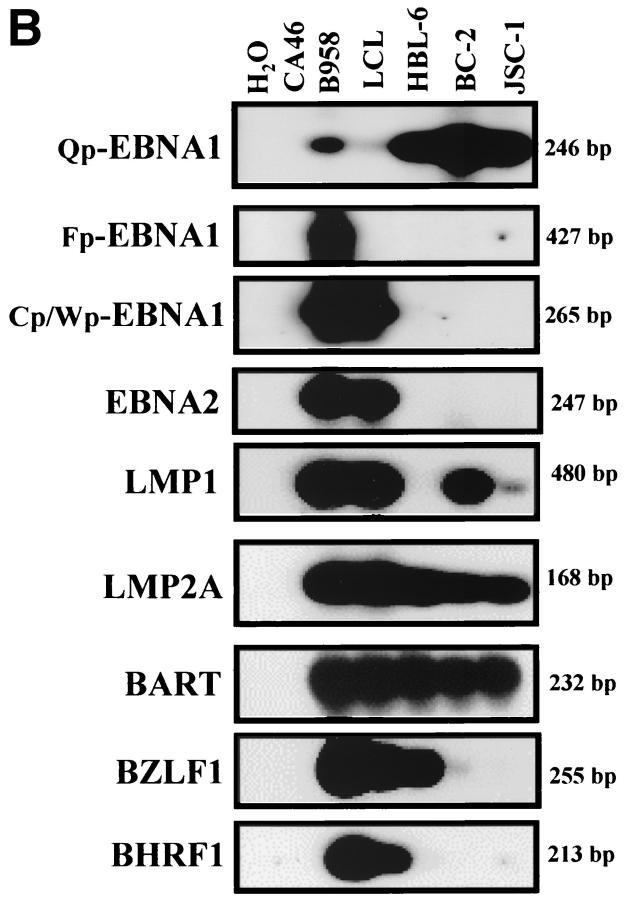
FIG. 1. EBV gene expression in PEL cells. (A) Immunohistochemical staining of EBV-encoded LMP1 and EBNA2 in JSC-1 and LCL cells. Note the absence of expression of LMP1 or EBNA2 protein in JSC-1 cells, whereas the cytoplasmic LMP1 and nuclear EBNA2 staining are readily demonstrated in LCL controls. Cells were counterstained with hematoxylin. Magnification, ×160. (B) RT analysis of EBV transcripts in EBV(+) PEL-derived cell lines: autoradiograph of a Southern blot showing hybridization of RT-PCR amplification products from HBL-6, BC-2, and JSC-1 cell lines. B95-8 and LCL served as positive controls, while CA46 served as a negative control. The H_2O sample contained no cDNA in the reaction.

tetradecanoyl phorbol acetate (TPA) (20 ng/ml) or butyrate (1 mM) for 36 h. Control cultures seeded and incubated in parallel were left untreated.

Immunohistochemistry and immunofluorescence. Immunohistochemical detection used monoclonal antibodies to EBV-encoded LMP1 (CS1-4; DAKO, Carpenteria, Calif.), EBNA2 (PE-2; DAKO), and ZTA (BZ-1; DAKO) and to KSHV-encoded LANA (LN53; from C. Boshoff, University College London, London, United Kingdom). Rabbit antisera were generated using synthetic peptides corresponding to amino acids 44 to 61 of KSHV open reading frame 21 (ORF 21) (vTK) and to amino acids 194 to 202 of KSHV ORF K2 (vIL-6) (5). Cytospin preparations of cells were fixed in a 1:1 mixture of acetone-methanol. Antibodies were applied at the following dilutions: anti-LMP1 (1:500), anti-EBNA2 (1:50), anti-ZTA (1:200), anti-vTK (1:500), anti-vIL-6 (1:1,250), and anti-LANA (1:500). For LMP1, EBNA2, and LANA immunodetection, a standard streptavidin-biotin technique with a horseradish peroxidase conjugate (DAKO) was used. For ZTA, vIL-6, and vTK, immunodetection was carried out with an alkaline phosphatase conjugate (Vector, Burlingame, Calif.). For immunofluorescence, cells were fixed in acetone and antibodies were applied at a 1:400 dilution for vIL-6.

In situ hybridization. A plasmid to generate riboprobes to the T1.1/nut1 KSHV lytic transcript (33, 34) was created by PCR amplification of BCBL-1 genomic DNA using T1.1-specific primers with the following sequences: 5′-GC ATTGGATTCAATCTCCAG-3′ and 5′-ACATCGTTAGTCAACCTAGC-3′. The amplification product was cloned in the sense and antisense orientations into the pCR 2.1 TA cloning vector (Invitrogen, San Diego, Calif.) downstream of the T7 RNA polymerase promoter, generating plasmids pJCT1.120A (antisense) and pJCT1.120S (sense). Plasmids to generate sense and antisense EBER riboprobes have been described previously (17). T1.1 and EBER plasmids were linearized and transcribed in vitro using digoxigenin-UTP (Boehringer Mannheim, Indianapolis, Ind.).

In situ hybridization studies were carried out on cells pelleted by centrifugation and fixed in 37% formalin. Cells were permeabilized with 0.3% Triton X-100 and digested with proteinase K (1 to 3 μg/ml) in 100 mM Tris-HCl and 50 nM EDTA at pH 8.0 for 15 min at 37°C. Heat-denatured digoxigenin-labeled riboprobes were applied to slides in a hybridization mixture containing 50% formamide, 10% dextran sulfate, 1% polyvinylpyrrolidine, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate, and 100 μg of salmon sperm DNA/ml in 5× SSPE (0.9 M NaCl, 50 mM NaH_2PO_4, plus 5 mM EDTA at pH 7.4). Specimens were hybridized for 16 h at 55°C in a sealed humidified chamber. Slides were washed successively in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× SSC, and 0.5× SSC for 1 h at room temperature followed by an incubation in RNase A (Boehringer Mannheim) at 10 μg/ml for 30 min. Hybridization was detected using an anti-digoxigenin antibody–alkaline phosphatase conjugate (Boehringer Mannheim).



Viral infection of DMVECs. PEL cells (JSC-1, BC-3, and BCP-1) were induced at 3×10^8 cells/ml with TPA at 20 ng/ml for 96 h. Cell-free culture supernatant (0.45 μM) was filtered and virus was pelleted by high-speed centrifugation for 2.5 h at 4°C at 20,000 × *g*. The virus pellet was resuspended in 2 ml of phosphate-buffered saline and used immediately to infect dermal microvascular endothelial cells (DMVECs) (approximately 80% confluent). DMVECs were seeded into two-chamber Lab-Tek slides (Rochester, N.Y.). Virus was added to cultures (25 μl/well) and incubated for 48 h. The medium was replaced every 48 h for the appropriate time (4, 9, 12, 15, and 22 days postinfection) for fixation in a 50:50 mixture of methanol-acetone (−20°C, 10 min). Slides were stored at −20°C for immunohistochemistry. Viral infection of DMVECs will be described in detail elsewhere (D. M. Ciufo, J. S. Cannon, L. J. Poole, F. Wu, P. Murray, R. F. Ambinder, and G. S. Hayward, unpublished data).

Quantitation of viral yields in the PEL supernatants was carried out by PCR amplification and compared to known copy numbers of cloned KSHV and EBV genes. For KSHV quantitation, T1.1-specific primers (see above) were used to amplify a 660-bp PCR product from DNase I-treated viral supernatants and from the T1.1 plasmid, pJCT1.120A, at various dilutions. PCR amplifications were optimized to detect as few as 10 copies of T1.1, involving initial denaturation at 95°C for 3 min, followed by 40 cycles consisting of 94°C for 30 s, annealing at 55°C for 1 min, and 72°C for 1 min, and then a final extension at 72°C for 10 min. The EBV Quantitative PCR Detection kit from Biosource International (Camarillo, Calif.) involving EBER I-specific primers was employed to amplify EBV sequences from DNase I-treated viral supernatants, DNA from infected DMVECs, and an EBER I control plasmid at various dilutions. PCR amplification was carried out according to the manufacturer's instructions and detected as few as 40 copies of EBER I.

RESULTS

Establishment and characterization of the JSC-1 cell line. The JSC-1 cell line was established from the ascitic fluid of a human immunodeficiency virus-seropositive, 52-year-old homosexual male who presented with a lymphomatous peritoneal effusion in the absence of a tumor mass. There was no previous

TABLE 1. Immunophenotypic profiles of a PEL cell line and the derivative cell line

Antigenic determinant	Presence of determinant in:	
	Lymphoma cells	JSC-1 cell line
CD10	+	ND[a]
CD19	−	−
CD20	+	+
CD22	−	−
CD25	+	ND
CD30	+	ND
Lambda Ig	+	−
CD3	−	−
CD5	−	−
CD33	−	−
CD45	+	+
CD71	+	+
HLA-DR	+	+

[a] ND, not determined.

history of Kaposi's sarcoma or other malignancy. Tumor cells were anaplastic, large, and hematopoietic in appearance. Cells from the resultant cell line, JSC-1, have a similar morphology, have a 48-h doubling time, and occasionally form clusters. The tumor and the cell line showed a similar phenotype by flow cytometry, with strong CD45 and CD71 expression, partial CD20 expression, and evidence of lambda light chain (Table 1).

Southern blot analyses of DNA extracted from the original lymphoma specimen and the resultant cell line showed immunoglobulin (Ig) heavy-chain rearrangements. Tumor specimen DNA showed two Ig heavy-chain gene rearrangements and background germ line material, whereas in the cell line DNA only one Ig heavy-chain rearrangement was preserved (data not shown). Cytogenetic analyses of the original tumor cells identified three distinct but related (all were +X) clonal populations (data not shown). The karyotype of the cell line after four months in culture was 45,XY,der(1)t(1;8)(q42;q11),add(6)(q25),-10,add(14)(q24).

EBV. PCR amplification of genomic DNA from the original tumor sample and the resultant cell line with primers specific to the EBNA-3C gene showed the presence of type I EBV (data not shown). As in previous reports of PEL cell lines, gene expression was highly restricted (15). LMP1 and EBNA2 were not detected by immunohistochemistry (Fig. 1A). By RT-PCR, Q promoter (Qp)-initiated EBNA1 transcripts were detected in JSC-1 and two other dually infected PEL cell lines (HBL-6 and BC-2; Fig. 1B). Neither Fp- nor Cp/Wp-initiated transcripts were detected in any of the three PEL cell lines, although these transcripts were detected in the control cell lines B95-8 and LCL. LMP1 transcription was detected by RT-PCR. The signal was intermediate in strength between the strong signal associated with BC-2 cells and the very weak signal (requiring overnight autoradiograph exposure) associated with HBL-6. LMP2A transcripts were detected in all three PEL lines, whereas no LMP2B transcripts were detected (Fig. 1B). *Bam*HI-A rightward transcripts were detected in the three PEL cell lines.

RT-PCR for two lytic EBV transcripts, BZLF1 and BHRF1, showed little or no expression (Fig. 1B). Among the three PEL cell lines examined, BZLF1 expression was weakest in JSC-1 and was only barely detectable. BHRF1 lytic transcripts were not detected in any of the PEL-derived cell lines. Both the original tumor and the resultant cell line were positive by EBER in situ hybridization (not shown).

KSHV. Sequence analysis of the highly variable K1 and K15 genes of KSHV identified the C3/P strain type (23, 35). Immunofluorescence showed very high basal vIL-6 expression in JSC-1 cells (30 to 35% of cells; Fig. 2, right panel) in comparison with the HBL-6 (<0.5%; Fig. 2, left panel), BCBL-1 (<2%), BC-2 (<0.5%), and BC-3 (<1.5%) cell lines (data not shown).

Pharmacologic induction of lytic gene expression. KSHV lytic gene expression was detected in a higher percentage of the JSC-1 cells than was observed for other PEL cell lines following treatment with chemical inducers, such as phorbol esters and sodium butyrate (16, 36). Representative data for the expression of the T1.1/nut1 lytic transcript in JSC-1 and BCBL-1 cells are shown in Fig. 3A. The levels of positive JSC-1 cells reached 40% at 36 h after butyrate treatment compared to 12% for BCBL-1 cells. Other putative lytic cycle genes (28), including vTK, G protein-coupled receptor, zinc finger membrane protein/immediate-early antigen (ORF K5), and ORF K8, similarly showed higher induction in JSC-1 cells in comparison with other cell lines (data not shown). Both early (<50 passages) and late (>300 passages) JSC-1 cells have reproducibly shown higher levels of lytic inducibility than any other PEL line tested (HBL-6, BCBL-1, BC-3, or BCP-1).

In contrast, expression of the KSHV TK occurred in a small percentage of untreated JSC-1 cells (less than 3%; Fig. 3B) but increased to approximately 50% of the cells following butyrate treatment (36 h) (Fig. 3C). The EBV-encoded immediate-early ZTA protein was not readily induced in JSC-1 cells following butyrate (Fig. 3D and E) or TPA treatment (data not shown).

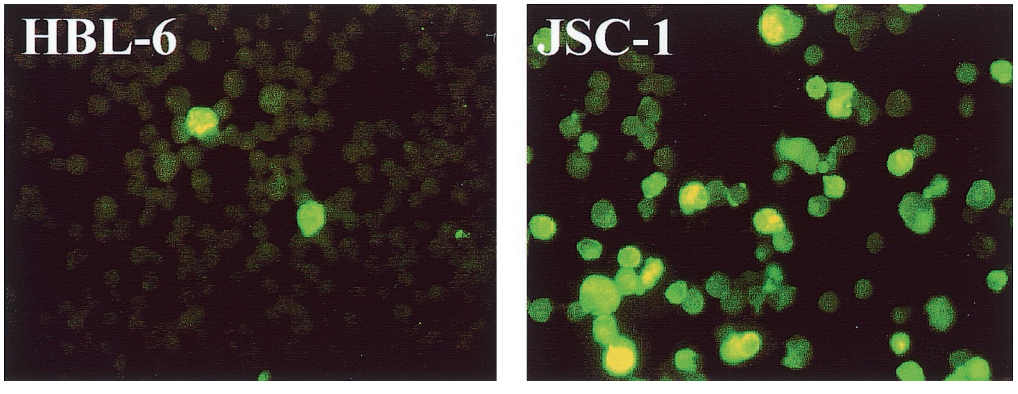


FIG. 2. High basal levels of vIL-6 expression in JSC-1 cells: immunofluorescence detection of vIL-6 in pelleted PEL cells of the HBL-6 and JSC-1 cell lines. Note 60-fold-higher expression of vIL-6 in JSC-1 cells than in HBL-6 cells.

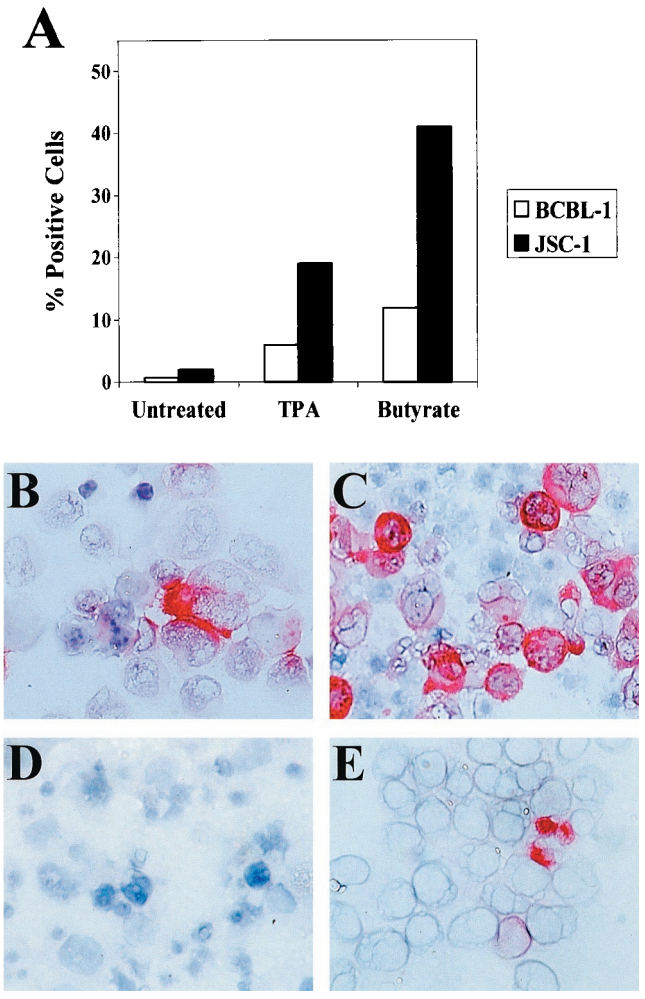


FIG. 3. Pharmacologic induction of KSHV lytic gene expression. (A) Expression of the T1.1/nut1 lytic gene transcript in JSC-1 and BCBL-1 cells with and without TPA or butyrate lytic inducing agents. Cells were treated with TPA (20 ng/ml) or butyrate (1 mM) for 36 h. Data represent positive stained cells, as determined by in situ hybridization with a T1.1 riboprobe. More than 10 fields of cells (>100 cells per field) were counted for each sample. (B) Immunohistochemical staining shows vTK expression in occasional JSC-1 cells in the absence of induction. (C) Following butyrate treatment, expression of vTK was detected in many more cells. (D) Immunohistochemistry for EBV-encoded lytic antigen ZTA in butyrate-treated JSC-1 cells shows no antigen expression. (E) ZTA expression shown for B95-8 control cells confirms positive ZTA antibody staining. An alkaline phosphatase detection system with a hematoxylin counterstain was used. Magnification, ×250.

Exposure to butyrate for greater than 40 h led to ZTA expression in less than 0.5% of the cells.

KSHV virion release and in vitro infection of DMVECs. Primary human DMVEC cell cultures displaying a contact-inhibited cobblestone shape acquired a spindle shape when exposed to JSC-1 supernatant (D. M. Ciufo, J. S. Cannon, L. J. Poole, F. Wu, P. Murray, R. F. Ambinder, and G. S. Hayward, unpublished data). The phenotype conversion was associated with LANA expression, which could be detected in foci of 2 to 12 adjacent cells as early as 4 days postinfection (Fig. 4). The number of LANA-positive cells per focus increased with time until 80 to 90% of the DMVECs were spindle shaped and LANA positive at 22 days postinfection. Infected endothelial cells did not become immortalized, senescing after 10 to 12 passages in culture unless fresh primary endothelial cells were added to the culture. We also tested, in parallel, the ability of viral supernatants from TPA-induced BC-3 and BCP-1 cell lines to infect DMVECs (Fig. 4). Spindle-shaped transformation and LANA expression were not detected until 22 days postinfection, and then they were detected only in very rare foci. Nine days postinfection with JSC-1 supernatant, spindle-shaped and mitotic cells were observed in every high-power field (Fig. 5). In mitotic cells, LANA expression showed spindle-shaped DMVECs undergoing chromosomal condensation, alignment, and separation at various stages of mitosis. However, mitotic figures were not seen following exposure to BC-3 or BCP-1 supernatant.

In order to assess whether the high efficiency of infection was due to larger quantities of KSHV virions in JSC-1 supernatant compared to supernatant from BC-3 or BCP-1 cells, we determined viral genome equivalents (vge) in each PEL supernatant by using quantitative PCR amplification after DNase I treatment and virion lysis. Supernatant from JSC-1 cells (2.5×10^6 vge/ml) contained greater than 2 orders of magnitude more DNase I-resistant KSHV DNA compared to BC-3 (2.5×10^4 vge/ml) or BCP-1 (2.5×10^6 vge/ml) supernatant virion DNA. DMVECs receiving 25 μl of pelleted virus from JSC-1 supernatant (6.25×10^4 virions) created 70 colonies of 2 to 12 LANA-expressing cells per colony by 4 days postinfection. In contrast, 25 μl of pelleted virus from BC-3 or BCP-1 supernatant (6.25×10^2 virions) resulted in one colony of six LANA-expressing cells (BC-3 supernatant) or in no colonies (BCP-1 supernatant) by 22 days postinfection. As described elsewhere (D. M. Ciufo, J. S. Cannon, L. J. Poole, F. Wu, P. Murray, R. F. Ambinder, and G. S. Hayward, unpublished data), these PEL supernatant preparations, when used in DMVEC infectivity assays, showed higher titers of infectious KSHV released in JSC-1 supernatant (3×10^3 PFU/ml) compared to BC-3 (30 PFU/ml) or BCP-1 (<10 PFU/ml) supernatants. Since JSC-1 cells are also infected with EBV, we assayed JSC-1 supernatant for levels of DNase I-resistant EBV virion DNA. JSC-1 supernatant contained 10- to 100-fold less EBV than KSHV virion DNA. Furthermore, no EBV DNA was detected in DMVECs infected with JSC-1 supernatant.

DISCUSSION

A new dually infected PEL cell line differs from previously characterized PEL cell lines (1, 7, 21, 25) in that there is high-level basal expression of KSHV lytic cycle gene products. Furthermore, filtered supernatant from TPA-treated JSC-1 cells efficiently induces morphologic transformation, LANA expression, and mitoses in primary human DMVEC culture.

Southern blot analysis of Ig heavy-chain rearrangements indicated the survival of only one clone of tumor cells since JSC-1 cells retained only one of the tumor-derived rearrangements. Similarly, cytogenetic analysis identified a single population of cells in the JSC-1 cell line that appeared to be a derivative of a minority clone of cells found in the original effusion lymphoma. Previously reported cytogenetic analyses of BC-1, BCBL-1, and BCP-1 cells (3, 30), along with the results presented in this report for JSC-1 cells, show that there are no consistent chromosomal changes observed among PEL cell lines. Likewise, no correlation could be made between specific chromosomal changes in the JSC-1 line and high basal lytic gene expression.

Analysis of EBV and KSHV strain types in JSC-1 cells shows that the cell line harbors strains of the two viruses similar to those found in other PELs (7, 35), and at least with regard to the types of strain variation examined, high-level basal and induced infection cannot easily be attributed to strain differences. This is consistent with our study of vIL-6 expression in

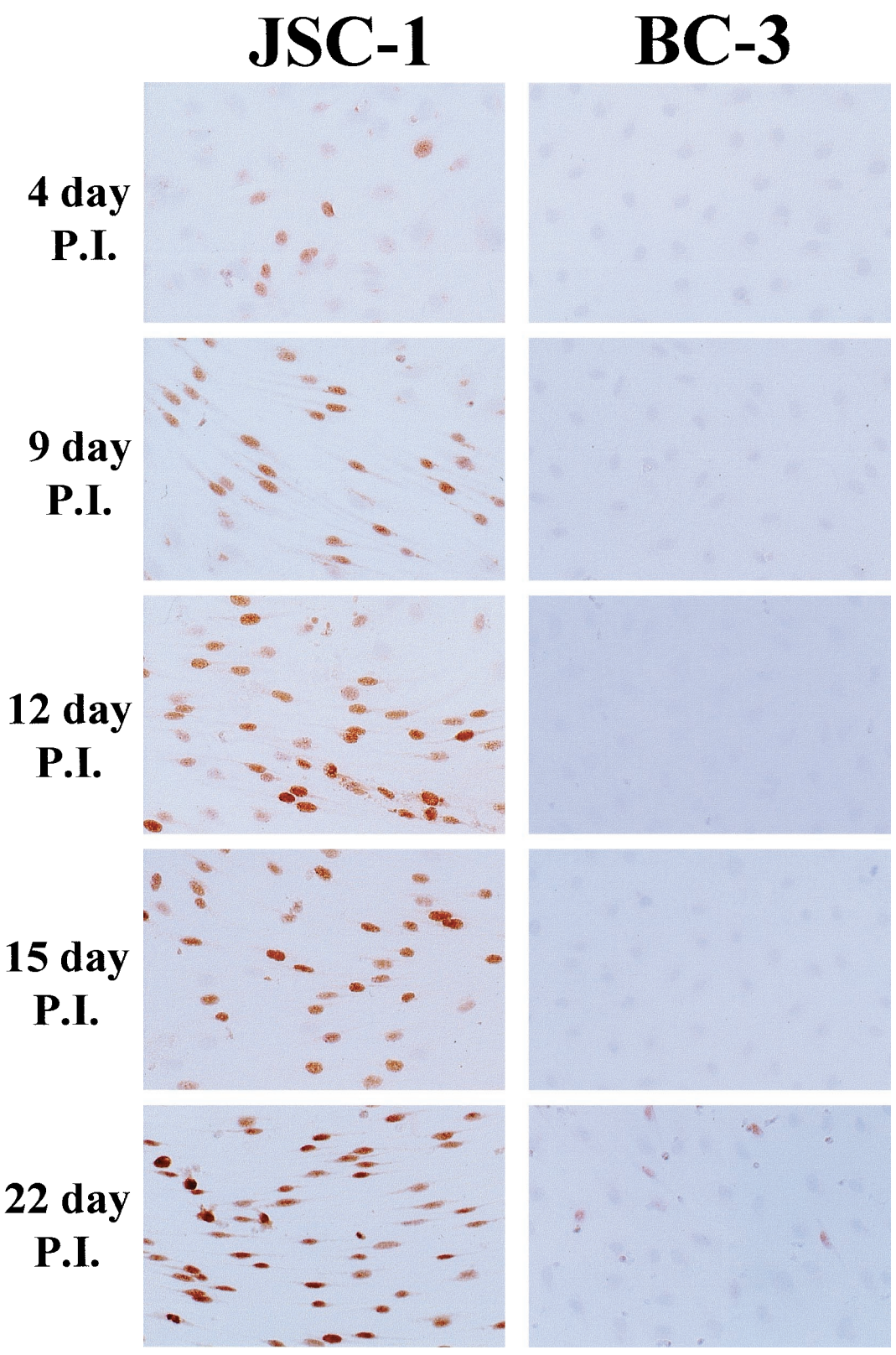


FIG. 4. Infection of DMVECs with viral supernatants of PEL cell lines: immunohistochemical staining of LANA in primary DMVECs following viral infection. Viral supernatants from JSC-1, BC-3, or BCP-1 (data not shown) cells were used to infect DMVECs that were seeded to dual-well culture slides. At 4, 9, 12, 15, and 22 days postinfection (P.I.), cells were fixed and analyzed for LANA expression. DMVECs infected with JSC-1 supernatant showed nuclear LANA staining at day 4. Note that the LANA-positive cells were still round at day 4. By day 9 postinfection, more cells expressed LANA, and these were spindle shaped. Supernatant from BC-3 and BCP-1 cell lines yielded only weak LANA expression and then were detected only at 22 days postinfection. Magnification, ×100.

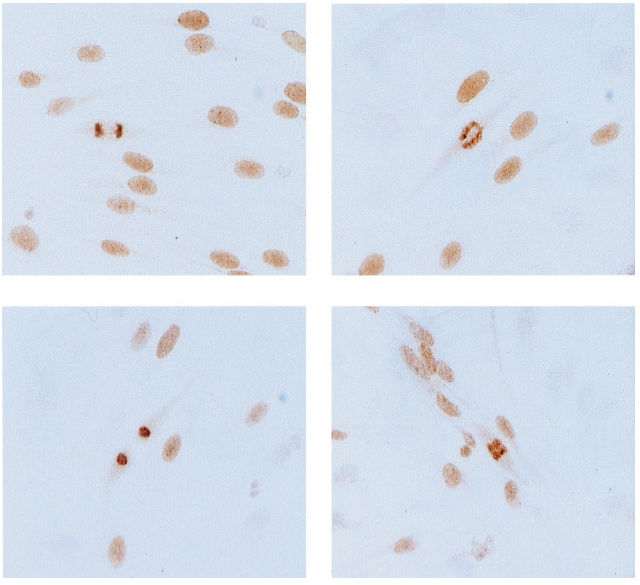


FIG. 5. LANA expression reveals mitotic changes in JSC-1-infected DMVECs. Immunohistochemistry for LANA expression shows spindle-shaped DMVECs undergoing chromosomal condensation, alignment, and separation at various stages of mitosis. These cells were identified as early as 9 days postinfection and could be detected at higher frequencies later after infection. Magnification, ×250.

rare Kaposi's sarcoma lesions that showed no association between levels of expression and viral strain (5).

EBV lytic gene expression was low in JSC-1 cells, in contrast to the very high basal expression of vIL-6 and higher-than-usual induced expression of other KSHV lytic genes. However, in all three PEL cell lines, EBNA1 transcripts derive from the Qp. No expression of EBNA1 or -2 was initiated from Cp or Wp. Varied LMP1 and BZLF1 expression was observed for the three PEL cell lines, with the weakest levels being detected in the JSC-1 cell line in both cases. LMP1 expression has been reported previously as being highly variable among different PEL tumors (15, 30). In contrast to LMP1, LMP2A was strongly positive for all three PEL lines. The *Bam*HI-A rightward transcripts are abundantly expressed and detected in all EBV-associated tumors and cell lines (9, 10) and were detected here in all three EBV(+) PEL cell lines.

Treatment with agents such as butyrate or phorbol esters causes a switch from latent to lytic KSHV infection in many PEL cell lines (18, 25). In JSC-1 PEL cells, lytic KSHV gene expression, but not EBV gene expression, was readily enhanced with TPA or butyrate. This pattern of differential inducibility of EBV and KSHV lytic cycle genes has previously been described for HBL-6 cells (18).

Supernatant virus from this cell line was much more efficient in producing a spindle phenotype, LANA expression, and generation of mitoses than was supernatant virus from BC-3 or BCP-1 (within 4 days for JSC-1 compared to 22 days for BC-3 and BCP-1). JSC-1 supernatant contains 100-fold more KSHV DNA than do BC-3 or BCP-1 supernatants, which likely contributes to JSC-1's higher efficiency of infectivity. This production of higher quantities of infectious virus from JSC-1 cells is in accord with the results shown in Figures 2 and 3, in which the JSC-1 cell line has a higher percentage of lytically induced cells than any other PEL line. Furthermore, JSC-1 cells contain much higher levels of KSHV DNA than BC-3, BCP-1, or BCBL-1 cells (measured by Southern blot hybridization). In addition to quantitative differences, it is also possible that the difference in infectivity is a result of qualitative differences in the virus produced from JSC-1 cells. Differences in lytic activation and virion production are well recognized among EBV-infected B-cell lines. For example, Akata cells are readily induced by surface Ig cross-linking (31), while B95-8 cells are readily induced only by TPA (19). Finally, although basal and induced EBV lytic gene expression were very low in JSC-1 cells, EBV virion DNA was detected in the JSC-1 supernatant. However, no EBV DNA was detected in DMVECs after infection with the JSC-1 supernatant. It is possible that the EBV virions in JSC-1 supernatant are defective or that, postinfection, EBV is lost from DMVECs. Whether or not the presence of EBV in JSC-1 cells and its supernatant contributes to the higher infectivity is not yet clear. In summary, the JSC-1 cell line, characterized by its high basal expression of some lytic antigens and high apparent inducibility of infectious virions, promises to simplify the investigation of KSHV infection in vitro, in the pursuit of a better understanding of the role of KSHV in the pathogenesis of PEL, multicentric Castleman's disease, and Kaposi's sarcoma.

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