Epstein-Barr Virus-Transformed B-Cell Line (DV-1) Derived from Bone Marrow of a Patient with Severe Combined Immunodeficiency and Immunoblastic Lymphoma

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ABSTRACT. An Epstein-Barr virus-transformed B-cell line derived from a patient with severe combined immunodeficiency who died of a lymphoreticular malignancy has been characterized. The line derived from bone marrow cultures and designated DV-1 shows surface and cytoplasmic IgM and staining with fluorescent monoclonal antibodies against immunoglobulin heavy and light chains μ, δ, and κ, Dr, and several other B-cell surface antigens. DV-1 secretes IgM_κ and demonstrates monoclonality on analysis of immunoglobulin heavy and light chain gene rearrangement patterns. Incubation with either phytohemagglutinin or pokeweed mitogen failed to cause significant stimulation of proliferation of DV-1 and another EBV-transformed B-cell line derived from an immunologically normal host (LA-350), whereas incubation with *Staphylococcus aureus* Cowan strain 1 led to significant inhibition of DV-1 and LA-350. Rabbit IgG antibody specific for human immunoglobulin μ-chains produced a dose dependent stimulation of both lines. The responses of DV-1 and LA-350 to mitogens and anti-μ were not as high as those of normal peripheral blood lymphocytes. This spontaneously derived Epstein-Barr virus-transformed B-cell line from a patient with severe combined immunodeficiency demonstrated functional characteristics similar to a B-cell line derived from an immune competent host. While these cells spontaneously incorporate 200 times more thymidine than normal resting peripheral blood lymphocytes, they retain their ability to be modulated by antiimmunoglobulin, and staphylococcal Cowan strain 1, but are unresponsive to the effects of B-cell growth factor. (*Pediatr Res* 21: 331–337, 1987)

Abbreviations

anti-μ, antiserum to IgM (μ specific)
BCGF, B-cell growth factor
EBV, Epstein-Barr virus

ELISA, enzyme-linked immunosorbent assay
FCS, fetal calf serum
HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
LBL, lymphoblastoid line
NK, natural killer cell
PBL, peripheral blood lymphocytes
PHA, phytohemagglutinin
PWM, pokeweed mitogen
R/H anti-μ, rabbit antihuman IgM (μ-chain specific)
SAC, *Staphylococcus aureus* Cowan strain 1 formalin fixed organisms
SCID, severe combined immunodeficiency
[^{3}H]dThd, tritiated thymidine
Fab'_2 anti-μ, antihuman IgM (μ-chain specific)
CALLA, common acute lymphocytic leukemia antigen

The polyclonal activation of immunoglobulin production and the proliferative transformation of a subpopulation of B-cells after infection with EBV has been known for some time (1). The morphological, proliferative, and functional characteristics of B-cell lines derived both spontaneously from EBV-infected hosts and by *in vitro* transformation of B-cells by certain strains of EBV have been reviewed by Nilsson and Klein (2). EBV transformation with malignant polyclonal and monoclonal proliferation of B-cells has been documented in a number of reports in both primary and secondary immune deficient hosts and has recently been reviewed (3).

We report initial studies characterizing a LBL derived from a 12-yr-old patient who died of an EBV-associated lymphoproliferative malignancy after bone marrow transplantation for SCID. The phenotypic and functional characteristics of this line are described and compared to another LBL derived from an immune competent patient (LA-350) and to normal peripheral blood lymphocytes.

MATERIALS AND METHODS

Cell lines. Cells were obtained from vertebral bone marrow at postmortem examination (approximately 1 h after death) and incubated for 24 h in modified Eagle's minimum essential media supplemented with penicillin and streptomycin, 10% heat inactivated FCS, and HEPES buffer. Subsequently, the cells were

cultured at 37° C in medium containing RPMI 1640 and 15% heat inactivated FCS with a bicarbonate-5% CO_2 buffer system. Unused aliquots of cells were frozen viably and stored in liquid nitrogen. Cell line LA-350 derived by EBV transformation of peripheral blood B lymphocytes from a patient with neuroblastoma prior to any therapy was the kind gift of Dr. R. C. Seeger (UCLA Center for Health Sciences, Los Angeles, CA) and was maintained in Waymouth's media supplemented with 15% FCS and antibiotics. PBL were obtained from normal adults and purified in a mononuclear cell suspension using a Ficoll-Hypaque gradient (4).

Cell surface antigen phenotyping. Nonsynchronous cell cultures were examined for a number of cell surface antigens defined by monoclonal antibodies obtained from commercial sources. Standard methods of indirect immunofluorescence followed by flow cytometry using a Coulter EPICS V and computerized analysis of the histograms by the IMMUNO program (Coulter Electronics, Hialeah, FL) was used to determine percentages of cells positive for each marker. A panel of monoclonal antibodies defining well accepted surface antigens commonly associated with certain functional and maturational features of T and B lymphocytes and NK was selected to characterize the DV-1 line. We routinely utilize most of these markers in characterizing the lymphocytes of our immunodeficient patients including the patient from whom DV-1 was derived (5). In addition the patterns of expression of these markers has been used to describe the phenotypic characteristics of a number of lymphoreticular tumors and cell lines. Monoclonal antibodies against the T-cell markers T3, T4, T8, T9, T10; the B-cell markers B1, B4, B7, DR (Ia), and immunoglobulin heavy (μ, γ, δ) and light (κ, λ) chains were obtained from Ortho Pharmaceuticals, Raritan, NJ. Monoclonal antibodies to B-cell markers B2 and Leu12 and NK markers Leu11, Leu7, and NKH-1; and myeloid markers J5 and M5 were purchased from Becton Dickinson Monoclonal Inc., Mountainview, CA. Monoclonal antibody 37.1 was produced against B-cells isolated from a patient with hypogammaglobulinemia and represents a middle maturation B-cell surface antigen expressed on CLL cells but not on ALL cells (6). Tissue typing was performed using standard microcytotoxicity methods by Dr. M. S. Pollack at Baylor College of Medicine Tissue Typing Laboratory.

Cytogenetics. Chromosome analysis was performed using G-banding techniques by Dr. D. Ledbetter at Kleberg Cytogenetics Laboratory, Baylor College of Medicine Houston, TX. Analysis was performed on several occasions using both mitogen stimulated and nonstimulated cells.

EBV detection. Infection of cells with EBV was detected by two methods. An anti-complement immunofluorescence assay was used by Dr. C. Sumaya, University of Texas, San Antonio, TX to detect the presence of EBNA (5, 7, 8). The quantitation of EBV genome in DNA isolated from DV-1 was performed using the standard slot blot technique and hybridization with a ^{32}P-labeled EcoRI-B fragment of EBV DNA provided by Dr. G. Miller (Yale University, New Haven, CT) (5, 9). This probe recognizes a noniterated sequence of the EBV genome.

Analysis of immunoglobulin gene rearrangements. DNA isolated from DV-1 was analyzed for heavy and light chain gene rearrangement using J_H heavy and κlight chain probes according to previously described methods (5, 10).

Immunoglobulin determination. Immunoglobulin synthesis was determined by ELISA assay of the culture supernatants according to published methods (11, 12). Briefly, either goat antihuman IgM, μ-chain specific or goat antihuman IgG, heavy and light chain specific (Cappel Laboratories, Malvern, PA) was coupled to polystyrene beads using a 0.1 M carbonate buffer pH 9.4 overnight at 4° C. The excess binding sites were saturated by incubation of the beads for 2 h at room temperature with a 1% solution of bovine serum albumin (Sigma, St. Louis, MO) in the same carbonate buffer. The antibody-bound beads were washed alternately with PBS pH 7.4 and 0.05% Tween-20 in PBS and

finally stored in a 1% solution of heat inactivated FCS in PBS with 0.05% sodium azide at 4° C until used. Solutions to be assayed were incubated in 50 μl volumes diluted with 200 μl of 1% BSA in PBS for 1 h at room temperature. Beads were washed with 0.05% Tween-20 in PBS and incubated for 1 h with alkaline phosphatase conjugated goat antihuman IgM (μ-chain specific) or antihuman IgG (heavy and light chain specific) antiserum (Cooper Scientific, Cochranville, PA). Beads were washed again and finally incubated for 30 min with 250 μl of 5 mM p-nitrophenylphosphate substrate (Sigma) in 1.0 M diethanolamine buffer at pH 9.8 (13). The reaction was stopped with 250 μl of 1.0 M NaOH and the samples brought to a final volume of 2.0 ml with PBS. Absorption at 405 nm was read using a Gilford spectrophotometer and the immunoglobulin content of the supernatants was determined from the standard curve generated using dilutions of normal human serm pools of known IgG, IgM, and IgA content.

Cell culture stimulation assays. Stimulation with the mitogens PHA (Burroughs Wellcome, Research Triangle Park, NC) and PWM (GIBCO, Grand Island, NY) was performed in 96-well microtiter plates according to published methods (14). Normal PBL control cultures consisted of 1 × 10^5 cells/well while cultures with LBL (LA-350, DV-1) contained 1 × 10^4 cells/well. Cells were cultured with 3 concentrations of PHA 10, 1, 0.1 μg/ml) and 3 dilutions of PWM (1:640, 1:2500, 1:5120) for 96 h at 37° C in 5% CO_2. The cultures were pulsed with [^{3}H]dThd (Amersham, Arlington, IL) at a final concentration of 10 mCi/ml for the last 20–24 h of incubation, harvested onto fiber filters using a multisample vacuum harvester, solubilized and suspended in scintillation fluid, and counted by liquid scintillation spectrometry. Control cultures were treated identically only with the mitogen omitted. In timed studies of PWM stimulation, cultures always were pulsed for the final 20–24 h but harvest times varied from 24 to 96 h.

Cells also were incubated with rabbit antibodies directed against IgM (μ-chain specific) with and without supernates from 72-h PHA-stimulated normal pooled PBL cultures. Sredni *et al.* (15) demonstrated the presence of BCGF activity in such supernates. Cultures were set up with 5 × 10^4 LBLs or 1 × 10^5 normal PBL. Cells were incubated for 48 h at 37° C with varying dilutions of either intact R/H anti-μ or rabbit (Fab')$_2$ anti-μ (Cappel Laboratories, Cochranville, PA). During the final 18–24 h, 2 mCi of [^{3}H]dThd was added to each 200-μl culture. Cells were harvested and counted by liquid scintillation spectrometry as above. Those cultures performed in the presence of BCGF contained a final concentration of 20% BCGF-containing supernate and were handled in the same manner. Cultures stimulated with SAC contained 1 × 10^5 normal PBL or 1 × 10^4 LBL (DV-1 or LA-350) and varying dilutions of SAC (Bethesda Research Laboratories Inc., Gaithersburg, MD.) either with or without 20% BCGF containing supernates. Cultures were pulsed as above during the last 24 h of incubation at 37° C in 5% CO_2 atmosphere and cells were harvested and counted in a liquid scintillation counter as above.

RESULTS

Establishment of cell line DV-1. Within the first 3–5 days of culture all bone marrow cultures contained a population of adherent fibroblast-like cells and a polymorphic population of nonadherent cells in suspension. Successful subculturing of nonadherent cells was initially dependent on the presence of this autologous adherent feeder layer. Commerical fibroblast (WI-38) monolayers were not capable of supporting growth of the nonadherent cells in suspension. After 6–8 wk of subculturing bone marrow cells with adherent feeder layers, the lymphoblastoid cells became capable of growth independent of the adherent cell layer. It is these bone marrow derived, feeder layer-independent cells which were termed DV-1 and were subsequently characterized.

Growth characteristics. DV-1 cells were pleomorphic and grew in suspension in large aggregates of cells easily disrupted by gentle pipetting. An adequate cell density was obtained by subculturing every 3–4 days at 1:4 to 1:10 dilution in fresh media. Nonsynchronous cell cultures had an approximate doubling time of 18–24 h and demonstrated a normal 46XY karyotype. Cells show small pseudopod-like projections on their surface when growing in suspension (Fig. 1) which is a commonly seen feature of transformed B-cells (2, 15). The LA-350 cells grow in similar aggregates, have a doubling time of approximately 12–18h, and demonstrate the same pleomorphism and membrane projections as described above.

EBV studies. Virtually 100% of the cells demonstrated EBV infection by immunofluorescence for EBNA. Quantitation of EBV DNA showed approximately 5–10 genome equivalents per DV-1 cell (Fig. 2).

Cell surface markers. Immunofluorescent staining with polyvalent antihuman Ig reagents showed positive fluorescence with typical capping and aggregation of surface immunoglobulins seen with peripheral blood B-cells. Frequency histograms of the cells stained for several common surface markers using indirect immunofluorescence and flow cytometry are illustrated in Figure 3. The cell line DV-1 appears to be a B-cell-derived line as there is no significant detection of typical T cell markers using monoclonal reagents and 97 and 91% of the cells were positive for Dr and B1 markers, respectively. Furthermore, the majority of cells bear IgM heavy chains and κ light chains on their surface while fewer than 20% show detectable IgG or λ light chains. In addi-
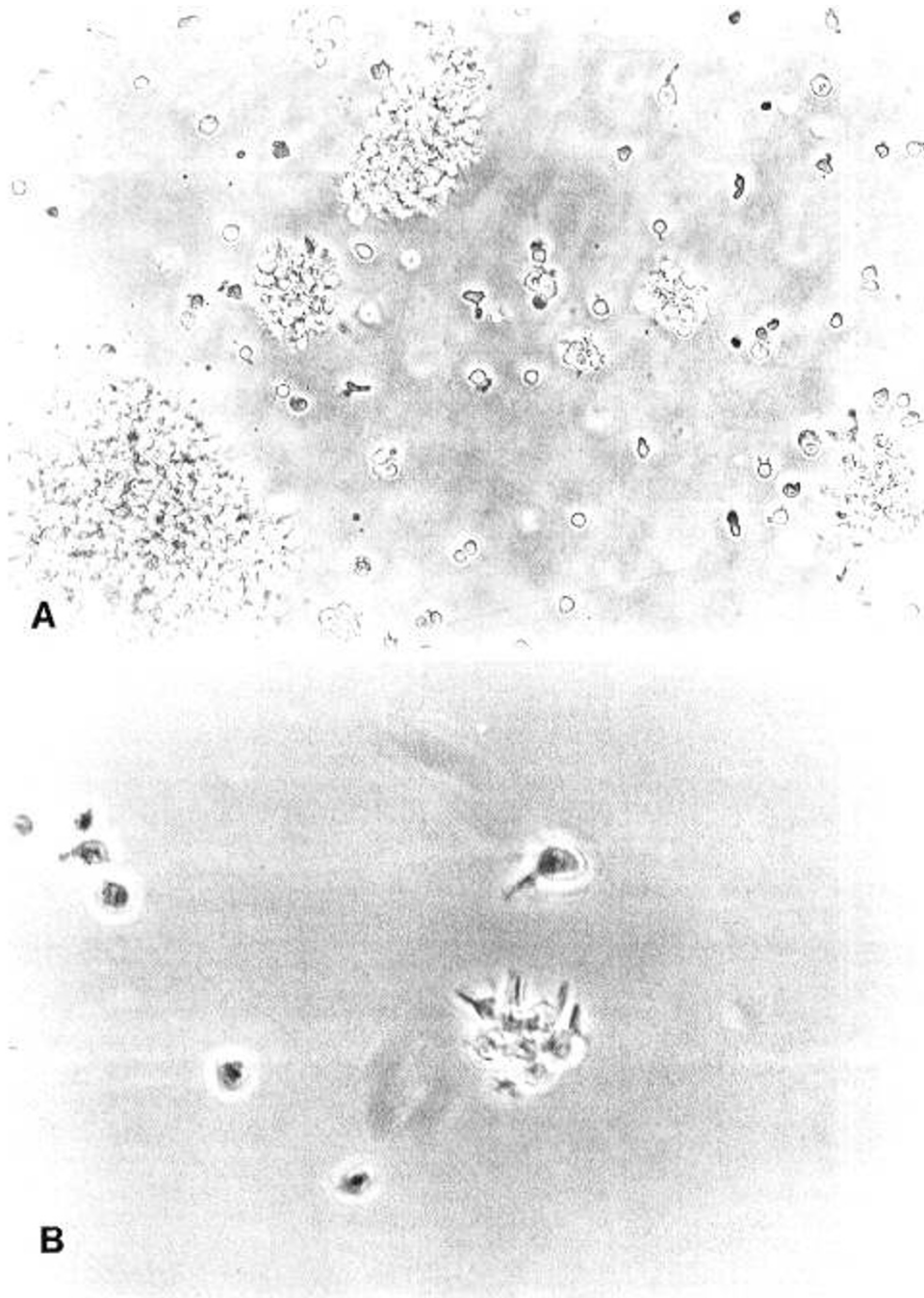

Fig. 1. Phase contrast photomicrographs of DV-1 cells demonstrating typical growth patterns in large and small cell aggregates (*A*) and pseudopod-like cytoplasmic projections (*B*). Photographs taken through a Nikon inverted phase contrast microscope with a Nikon HFM automatic metering system at 100× and 200×, respectively, using Kodak Plus X Pan ASA 125.

Fig. 2. Slot blot analyses of EBV DNA from the DV-1 cell line and control specimens. Five µg of DV-1 DNA and human sperm DNA (*C*) were applied to the membrane. Different amounts of EBV DNA corresponding to varying numbers of genome equivalents, as indicated by *numbers* in the *figure*, were also applied to the membrane for quantitation of the results.
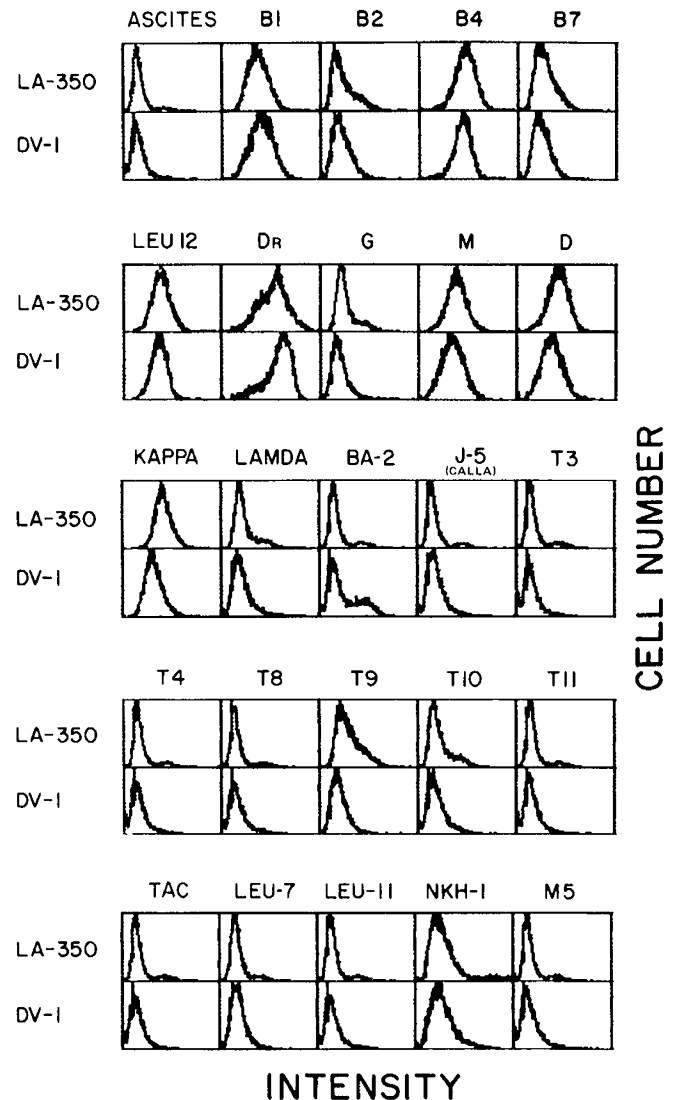

Fig. 3. Immunofluorescence histograms for cell surface markers on LA-350 and DV-1 cells using FITC conjugated monoclonal antibodies as described in "Materials and methods." A total of 20,000 cells was counted for each surface marker.

tion, 85% of unsynchronized cells express IgD on their surfaces. Analysis for the presence of a number of other monoclonal antibody-defined surface antigens demonstrated the absence of myeloid markers, J-5 or CALLA, the monocyte marker M-1, and the NK markers detected with two monoclonal reagents (leu 7 and leu 11). The intensity shift of the entire population with NKH-1 staining probably represents nonspecific Fc binding and not true expression of the NKH-1 antigen.

Percentages of these markers on the LBL lines DV-1 and LA-350 are summarized in Table 1. It is interesting that the premortem PBL of the patient displayed marked reactivity with B1, Dr, T10 and NKH-1 antibodies (5).

Gene rearrangement studies. Autoradiograms for both immunoglobulin heavy chain and κ light chain gene rearrangements (Fig. 4) each showed one germline band and one clonally rearranged nongermline band. The findings of a single intense rearranged band for both heavy and light chain genes and the equal intensity of the germline and rearranged bands suggest the presence of a monoclonal population of DV-1 cells in which one allele is rearranged and the other is not. Interestingly, the patterns noted in the autoradiogram from DV-1 did not match the patterns of any of the tumors found at postmortem examination of the patient from whom the line was derived. Two monoclonal lesions found in the lung and jejunum showed only κ light chain gene rearrangement. Lesions from several other locations showed oligoclonal J_H banding patterns and evidence for rearrangement of κ and λ genes (5).

Proliferative studies. The dose-dependent proliferative response of DV-1, LA-350, and control PBL to PHA and PWM is illustrated in Table 2. Compared to the usual proliferative responses of normal PBLs to these mitogens, only a minimal response was seen in either DV-1 and LA-350, respectively, at the highest concentration of PHA. These trends began as early as 48 h incubation and were still detectable after incubation for 96 h with the appropriate mitogens. To determine if there was a time-dependent effect of PWM on these cells, incubations were carried out using the highest PWM concentration (1:640 dilution) for various lengths of time from 24 to 96 h. The results (data not shown) indicate minimal PWM responsiveness of both LBLs at all time points.

Effect of R/H anti-µ. Preliminary titration studies using various concentrations of anti-µ showed optimal stimulation of both PBL and LBLs at 48 h (data not shown). Incubation of PBL and LBL for 48 h with anti-µ at several concentrations from 0–175

µg/ml produced a dose-dependent stimulation of thymidine incorporation of all three cell types (Table 3). This effect appeared to be specific since previous studies utilizing irrelevant proteins such as control rabbit antiserum, murine monoclonal antibodies against human Thy-1, and a human neuroblastoma associated surface antigen caused no alteration in cell proliferation (data not shown). From the data shown in Table 3 it appears that while the BCGF containing supernatant enhanced the proliferative effects of anti-µ on PBL by 40 to 240% at different concentrations of anti-µ; no such enhancement was seen for DV-1 or LA-350. There was not a significant difference between stimulation with the intact Ig molecule as compared with the divalent F(ab')₂ fragment (data not shown). Interestingly, at the lower concentrations, stimulation was more consistent for the LBLs than for normal PBLs.

Proliferative effects of SAC. Preliminary studies established that maximal [³H]dThd incorporation by normal unseparated PBLs occurred at 96 h and by LBL at 72 h over a wide range of dilutions of SAC. The proliferative effects of SAC on PBL, DV-1, and LA-350 over a wide range of dilutions at 72 h are shown in Table 4. The typical stimulatory effect of SAC is demonstrated for PBL with a 6-fold increase in [³H]dThd uptake at a 1:5000

Table 1. *Phenotypic Characteristics of DV-1 and LA-350**

B-cell markers:	DV-1	LA-350	T-cell markers:	DV-1	LA-350	Other:	DV-1	LA-350
B1	91	93	T3	2	6	J-5	11	5
B2	27	32	T4	2	5	M5	8	5
B7	39	55	T8	4	4	BA-2	33	9
B4 (Leu 12)	96	97				37.1	72	70
						TAC	2	7
Ia (Dr)	97	97	NK markers:			T9	16	61
IgG	15	16	Leu 7	12	7			
IgM	83	93	Leu 11	4	4			
IgD	85	95						
κ	84	98						
λ	20	16						

* 10^4 cells stained with each monoclonal antibody compared with control second step stained cells. Values represent the percent of the total mononuclear cell fraction positive for each respective marker. Population shifts of greater than 20% were required before considering a cell positive for any marker.
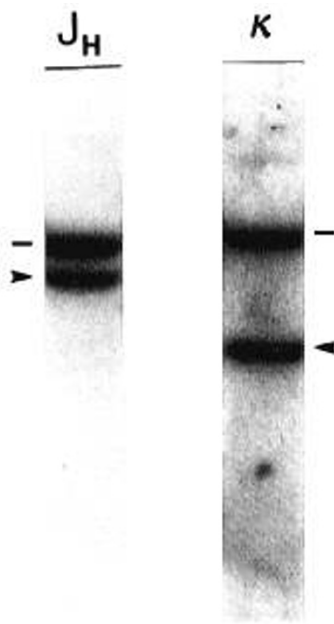

Fig. 4. Autoradiograms of immunoglobulin gene rearrangement analyses. On the *left* is shown the analysis of immunoglobulin heavy chain DNA using the J_H hybridization probe; on the *right* is the analysis using the κ light chain probe. *Dashes* indicate germline bands and *arrowheads* indicate clonally rearranged bands. Both analyses were performed after digestion of DV-1 DNA with Bam HI-restriction enzyme.

dilution. By contrast both DV-1 and LA-350 showed a substantial dose dependent inhibition of [³H]dThd uptake in response to incubation with SAC. Maximal inhibition occured at a 1:50 dilution of SAC with 75 and 73% inhibition for DV-1 and LA-350, respectively. It should be noted that baseline [³H]dThd incorporation for unstimulated LA-350 under these culture conditions is 200-fold greater and for DV-1 10-fold greater than baseline for unstimulated PBL.

Other studies. Immunoglobulin secretion of DV-1 and LA-350 was determined by quantitation of supernatants from cultures intially seeded at a cell density of 5×10^4 cells/ml in Costar 25-mm flasks. Samples were harvested by centrifugation at 1000 × g for 10 min at varying incubation times and supernatants were stored at −20° C until assayed. Using the ELISA methods described above no IgG was detected and the IgM content of DV-1 and LA-350 supernatants ranged from 900 to 1200 ng/ml and 200 to 400 ng/ml, respectively, depending on the culture conditions. No specific binding activity for either EBV antigens by indirect immunofluorescence (Dr. Ciro Sumaya, San Anto-

nio, TX) or for neuroblastoma surface antigens by radioimmunoassay (16) was noted in respective supernatants.

DISCUSSION

The clinical data on the patient from whom this cell line was produced have been reported recently (5, 14). To summarize his immunological status, he had always shown absolute lymphopenia generally with absolute counts of approximately 400 cells/ mm³. Total T-cells by E rosettes usually ranged from 5–15% (normal 43–81%) and sIg+ lymphocytes were usually elevated, ranging from 15–49% (normal 6–18%). Most of the B-cells stained for IgM and IgD but lacked IgG and IgA. Monoclonal antibody staining of surface markers showed T3, T4, T8, and T11 consistently below 25, 14, 10, and 23%, respectively, for at least 2 yr prior to transplantation. The B-cell markers Ia1 and B1 were usually elevated at 68 and 45%, respectively. Functionally, serum immunoglobulin levels for IgG, IgA, and IgM were consistently <10, 20, and 50 mg/dl, respectively, with no evidence for specific antibody production to diphtheria, tetanus, or blood group antigens. *In vitro* proliferation studies to PWM, PHA, Con-A, and several bacterial and fungal antigens was consistently depressed, but MLC reactivity was present.

DV-1 shows many of the characteristics that have been attributed to EBV-derived LBLs. This line arose spontaneously with a relatively short delay period (only 2–3 wk) and cell growth was dependent on the presence of a "feeder" layer for 60–90 days. Subsequently the line grew independently in suspension demonstrating the typical pattern of growth and "classic" lymphoblastoid morphology. The cells were pleomorphic in culture and had a baseline [³H]dThd incorporation 60–100 times that of normal unstimulated PBL. The presence of predominantly IgM_κ with some expression of IgD by these cells and the absence of chromosomal abnormalities distinguish them from most Burkitt's lymphoma lines and other clonally transformed non-Hodgkin's lymphoma cell lines. The B-cell origin of these cells is suggested by the presence of membrane bound IgM and IgD, Dr, B4, B7, B1, and 37.1 surface markers and the absence of the common T-cell markers T3, T4, T8, and T11. The cells were not responsive to stimulation with either PHA or the T-cell-dependent PWM, whereas B-cell activators SAC and antiserum to μ-heavy chains showed significant inhibitory and stimulatory effects, respectively. Both LA-350 and DV-1 cells differed from PBL in their lack of enhancement of anti-μ-induced proliferation by the BCFG-containing supernatant. Although there appeared to be some reversal of the anti-μ-stimulated proliferation of LA350 by BCGF, the DV-1 cells showed no such reversal in the presence of BCGF.

The host derivation of DV-1 was confirmed by the 46XY karyotype and the HLA phenotype. Virtually 100% of these cells were positive for EBNA on fluorescence and the presence of

Table 2. *Spontaneous and induced proliferation of PBL and LBLs*

Cell type Mitogen: Concentration:	CPM [³H]dThd incorporated							
	PHA				PWM			
	None	0.1 μg/ml	1 μg/ml	10 μg/ml	None	1:5120	1:2560	1:640
PBL	1558 ±88	5442† ±1340	216501† ±8641	267022† ±10757	2135 ±218	30122† ±2143	37271† ±1655	42389† ±2014
DV-1	40217 ±1321	38568 ±3442	35450‡ ±1437	24866† ±1190	41244 ±1293	40445 ±2459	42148 ±1562	42531 ±872
LA-350	310825 ±5476	264911 ±48934	275976‡ ±16747	256333† ±6733	289736 ±12941	288763 ±8441	293698 ±13893	311567 ±7820

* Incorporation of [³H]dThd after a 72-h incubation of each cell type with the specified concentrations of mitogen. Initial cell numbers were: PBL—1 × 10⁵, DV-1 and LA-350—1 × 10⁴ cells/culture. Data are from a representative experiment performed in quadruplicate.
† $p \leq 0.001$.
‡ $p \leq 0.01$.

Table 3. *Effect of anti-μ on proliferation**

Cell type	BCGF	Anti-μ concentration (μg/ml)					
		0	5	11	22	44	175
LA350		140,800 ±1,092	149,106 ±7,187	155,173† ±7,762	151,694‡ ±2,885	135,797 ±8,490	119,238‡ ±5,774
DV-1		19,523 ±536	30,940‡ ±1,192	30,647‡ ±1,826	29,037† ±4,122	27,321† ±2,993	24,709‡ ±2,856
PBL		1,209 ±156	3,949‡ ±503	2,522† ±437	1,935‡ ±126	1,511 ±525	882 ±102
LA350	20%	141,134 ±818	142,807 ±6,484	141,548 ±3,754	137,346 ±4,328	138,905 ±2,463	133,936 ±4,599
DV-1	20%	19,368 ±857	30,909‡ ±1,789	28,475‡ ±2,024	27,918† ±3,050	27,523† ±2,962	26,281‡ ±1,619
PBL	20%	1,970 ±254	6,179‡ ±670	3,691‡ ±601	3,606‡ ±194	4,448 ±1,954	2,994† ±478

* Values represent mean cpm ± SD. [³H]dThd incorporated by 1 × 10⁵ PBL or 1 × 10⁴ LBL from 24–48 h with varying concentrations of rabbit F(ab')₂ anti-IgM (μ-specific) in the presence or absence of BCGF. Values represent normalized data from two experiments with overlapping concentration ranges run in quadruplicate.
† $p \leq 0.01$.
‡ $p \leq 0.001$.

Table 4. *Effect of SAC on proliferation**

SAC final dilution	LA-350	DV-1	PBL
(Media only)	310,882 ± 6,663	24,780 ± 602	1,647 ± 222
1:50	86,126 ± 3,414	6,677 ± 698	5,084 ± 158
1:100	147,501 ± 5,117	11,812 ± 1,232	9,835 ± 548
1:500	278,854 ± 8,436	19,255 ± 2,048	15,204 ± 427
1:1000	264,721 ± 13,605	20,256 ± 937	14,877 ± 549
1:5000	255,374 ± 12,329	19,166 ± 480	15,335 ± 811

* Values represent mean cpm ± SD and normalized to the mean of values for overlapping dilutions of SAC for two experiments run in quadruplicate. $p \leq 0.001$ for all values compared to unstimulated control cpm.

EBV DNA was confirmed by hybridization studies. The finding of 5-10 EBV genome equivalents per cell and the spontaneous *in vitro* production of 900–1200 ng/ml of IgM are comparable with other EBV transformed LBLs which have been studied (2).

Two features distinguish DV-1 from most other LBL. Outgrowth of DV-1 showed a 2- to 3-wk "lag" period. Although somewhat subjective this is clearly different from the 30–100 days reported for most LBL from infectious mononucleosis patients and more closely resembles outgrowth of Burkitt's lymphoma lines (15). Abnormal T-cell restraint of rapid proliferation of nonpermissively EBV-infected B-cells may have encouraged the early outgrowth of DV-1 (17, 18).

The typical polyclonal LBLs arising from B-cells infected with EBV naturally or *in vitro* may become monoclonal after periods

of 1 yr or longer (2, 19). The monoclonality of DV-1 suggested by the virtual absence of λ light chain and confirmed by Ig gene rearrangement (Fig. 4) was detected in the 2nd month of culture. Early monoclonal restriction might be explained by the preexistent B-cell defect demonstrated in 1979 prior to EBV exposure (14), the lack of normal T-cell control of isotype diversity, or the selective *in vitro* outgrowth of B-cells present in the marrow which were already monoclonally transformed but not detectable grossly or microscopically.

The stage of maturation of DV-1 may be analyzed by the expression of various surface differentiation antigens. The typical B-cell differentiation antigens B1, B4, and B7 as well as coexpression of IgM and IgD imply differentiation to a mature but "naive" B-cell. Moreover, the B2 marker, associated with the EBV receptor and demonstrated to show activation and cell cycle-dependent expression (20), was demonstrated on roughly 30% of unsynchronized cells.

The correlation between EBV immortalization and immunoglobulin secretion has been demonstrated in two recent reports (21, 22). While it is clear that only about 50% of normal B-cells capable of stimulated immunoglobulin secretion are immortalized by EBV (21), it is not clear what relationship the pattern of antigenic expression on DV-1 has with respect to its functional properties of spontaneous IgM secretion or the specific, although modest, modulation of proliferative activity of LA-350 and DV-1 by SAC and anti-μ. These findings are compatible with functional studies on malignant B- and T-cells reported by other authors (23, 24). Such modest effects on DNA synthesis and cell proliferation triggered by activation events at the cell membrane have been associated with dramatic effects on cellular metabo-

lism and constitutive synthesis of a number of glycoprotein receptors and phospholipid turnover (25–28).

It would appear from our preliminary observations that these LBLs may be subject to some of the same regulatory mechanisms as normal B-cells, and further study of this LBL model system holds significant potential for the effective production of human monoclonal antibodies by LBL, the elucidation of some mechanisms involved in viral B-cell activation, and the clarification of some intrinsic B-cell defects present in patients with humoral immunodeficiencies.

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