

Spontaneous Production of High Levels of Leukocyte (α) Interferon by a Human Lymphoblastoid B-Cell Line (LDV/7) (41486)

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Abstract. A human lymphoblastoid B-cell line (LDV/7) capable of replicating significant levels of transfer factor and immune RNA was found to produce high quantities of interferon continuously. The maximum yield of interferon (600 units/ml) was detected on the seventh day. The interferon was characterized as human leukocyte (α) type. This interferon exhibited broad antiviral activity against vesicular stomatitis virus, poliovirus, sindbis virus, *Herpesvirus saimiri*, and Epstein–Barr virus. Treatment of LDV/7 cells with TPA, PHA, or LPS failed to enhance interferon production. Furthermore, LDV/7 cells could not be induced with Sendai virus to produce larger amounts of interferon. Interferon production correlated best with cell proliferation. It is therefore possible that this cell line may be a good source for large-scale production of human leukocyte interferon as well as for cloning of the interferon genome, and for studies of gene expression.

A human lymphoblastoid cell line (LDV/7), established in 1968 from the peripheral blood leukocytes of a healthy 65-year-old male, has been shown to be the only lymphoblastoid cell line capable of replicating significant quantities of transfer factor (TF) and immune RNA (1, 2). Other human lymphoblastoid cell lines (B or T), or cell lines established from Burkitt's lymphoma (3, 4), including Namalwa (3), were found to be unsatisfactory for replication of TF. Since LDV/7 is a B-cell line, possessing a unique ability to replicate TF, we were interested: (a) in investigating its ability to produce interferon spontaneously or by induction with Sendai virus or other viruses, as has been reported previously for other lymphoblastoid cells (5–8), and (b) in testing the antiviral activity of this interferon, particularly on two primate oncogenic herpesviruses, Epstein–Barr virus (EBV) of man and *Herpesvirus saimiri* (HVS) of the squirrel monkey.

Materials and Methods. The characterization of LDV/7 cells, including sur-

face markers, cytochemistry, immunoglobulin secretion, HLA type, chromosome analysis, presence of EBV DNA and antigens, and induction of tumors in athymic nude mice has been reported elsewhere (9). LDV/7 cells were grown at 37° in RPMI–1640 medium (Gibco) containing 10% heat-inactivated fetal bovine serum and antibiotics. Unlike some B-cell lines, LDV/7 cells are unique in their growth pattern since more than 15% of the cells firmly attach to the plastic surface, and clumps of cells, both attached and floating, are consistently present in culture.

Treatment of cells for interferon production. LDV/7 cells were seeded at 2×10^5 cells/ml in RPMI 1640 medium containing 10% fetal calf serum. At the time of seeding >95% cells were viable. These cells were divided into various groups, as described below, for treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) and Sendai virus. Supernatants were harvested at various times and were clarified at 300g, filtered through a 0.45- μ m filter and frozen in liquid nitrogen. The procedure was as follows:

1. LDV/7 cells supernatants harvested at 3, 7, 10, and 14 days;

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2. LDV/7 cells treated with 20 ng/ml of TPA for 3, 7, and 14 days and supernatants harvested as for untreated LDV/7 cells;

3. LDV/7 cells infected with 4 HA unit of Sendai virus and supernatants harvested as for TPA-treated cells;

4. LDV/7 cells treated with phytohemagglutinin (PHA-P), 2 μ g/ml and *Escherichia coli* lipopolysaccharides (LPS), 25 μ g/ml; and

5. LDV/7 cells treated first with PHA-P (2 μ g/ml) and then with 20 ng/ml of TPA.

Assays for interferon. The assays were performed by the VSV plaque reduction method as described previously (10, 11). The titers are expressed as international reference units per milliliter.

EBV and HVS sensitivity to LDV/7-produced interferon. This was carried out according to the methods previously described (12). The Ag-876 strain of EBV from cells grown at 34° was used for induction of EBV capsid antigens (VCA) and transformation of human cord blood lymphocytes (HCBL) (13). The titer of this virus had previously been shown to be 10⁶ transforming units/ml in HCBL.

Results. Growth characteristics of LDV/7 cells. LDV/7 cells form clumps consisting of 5–400 cells. These clumps either attach to the plastic surface or float in the culture medium. On subculture, the floating cells and/or attached cells were found to attach to the plastic surface as well as float in the medium. LDV/7 cells replicate faster than Raji, Ag-876, P3HR-1 cells, and Namalwa (6).

Interferon production. The spontaneous levels of inhibitor detected in the supernatants of LDV/7 cells at various times are presented in Fig. 1. The peak of inhibitor production (approximately 600 units) was on the seventh day and thereafter the level of interferon declined.

The LDV/7 cells treated with TPA showed only slight enhancement of inhibitor production by 0.2₁₀ (Fig. 1) on Day 7; however, on Day 3 approximately 500 units of interferon were detected in TPA-treated cells (an increase of 380 units). On Day 14, TPA-treated samples showed an increase of only 30 units over the spontaneously produced inhibitor.

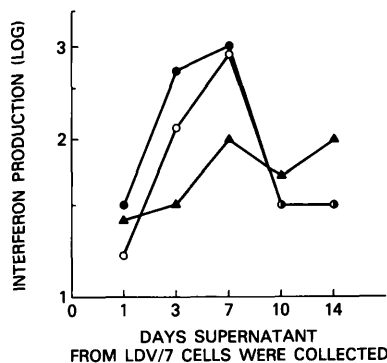


FIG. 1. Detection of interferon (log) in LDV/7 lymphoblastoid cell cultures in the presence and absence of TPA and Sendai virus. ○, LDV/7 supernatant only; ●, LDV/7 supernatant with the presence of TPA; ▲, LDV/7 supernatant harvested after infection with Sendai virus.

The Sendai virus infected LDV/7 cells showed a significant drop in the level of the inhibitor (Fig. 1) indicating that LDV/7 cells contain a considerable level of inhibitor used to neutralize the virus.

LDV/7 cells treated with 2 μ g/ml of PHA-P or 25 μ g/ml of LPS for 3 and 7 days failed to induce any increase in the level of spontaneously produced inhibitor (\leq 120 units Day 3 and \leq 400 units Day 7). However, the treatment of LDV/7 cells first with PHA and then with TPA resulted in producing \geq 600 units of the inhibitor, but this effect decreased significantly by Day 7 (\leq 160 units).

Characterization of the inhibitor produced by LDV/7 cells. As presented in Table I, the inhibitor was characterized as being interferon. The inhibitor showed the following major properties of human leukocyte interferon: (a) the inhibitor was active against different viruses: sindbis virus, poliovirus, and vesicular stomatitis virus; (b) the inhibitor was species specific in that it protected human WISH and human Hep-2 cells but it did not protect mouse L cells against virus infection; (c) the inhibitor was stable to acid treatment (pH 2) at 4° for 24 hr; (d) the antiviral activity was cell mediated since the washing of the cells treated for 24 hr with the inhibitor did not remove the resistance to virus infection; (e) the inhibitor was inactivated by antiserum

TABLE I. CHARACTERIZATION OF VIRUS INHIBITOR^a

Sample treatment	Cell type: Virus:	Human	Human	Human	Mouse	pH 2
		WISH	WISH	Hep-2	L	Human
		VS	Sindbis	Polio	VS	WISH
		VS	VS	VS	VS	VS
LDV/7 supernatant harvested Day 3		120	>100	>100	—	100
LDV/7 supernatant harvested Day 7		600	>100	>100	<3	100
LDV/7 cells treated with 20 ng/ml of TPA supernatant, harvested Day 3		500	>100	>100	—	—
LDV/7 cells treated with 20 ng/ml of TPA supernatant, harvested Day 7		1000	>100	>100	<3	100
LDV/7 cells infected with 4 HA units of Sendai virus, supernatant harvested Day 7		500	>100	>100	—	—

^a Data are expressed in international reference units/ml.

Inhibitor characteristics: (1) Broad antiviral activity; (2) cell species specificity; (3) stable at pH 2; (4) the inhibitor is cell associated since the inhibitor is not removed after three washings of the cells before virus challenge; (5) the inhibitor was destroyed by trypsin and not by DNase or RNase (data not shown); (6) the inhibitor was not sedimented by ultracentrifugation.

directed against leukocyte interferon (Table II); and (f) the inhibitor was destroyed by trypsin but not by DNase or RNase and was not sedimented by ultracentrifugation.

Antiviral activity. Results of LDV/7 lymphoid cell interferon on oncogenic HVS of nonhuman primates and EBV are presented in Table III. Continuous post-treatment of owl monkey kidney (OMK) cells with LDV/7 supernatant harvested Day 7 reduced the HVS titers by 3 logs. If the OMK cells were pretreated with the interferon, a further half-log reduction in HVS titer was observed.

The interferon from LDV/7 cells failed to induce any effects on intrinsic EBV genome expression in Raji cells, since no reduction or inhibition of spontaneous expression of EBV nuclear antigen (EBNA) was observed. This interferon also failed to block VCA-EA expression in EB virus producers Ag-876 or P3HR-1 cells (Table III). Superinfection of Raji cells with lytic P3HR-1 EBV normally leads to expression of early antigens (EA). Table III shows that after superinfection of Raji cells with 10^{4.0} EA inducing units, approximately 10% EA were detected by indirect IF. More than

TABLE II. ANTIGENIC TYPING OF LDV/7 INTERFERON^a

Interferon sample	Medium	Antiserum	
		Anti-fibroblast (β) IFN	Anti-leukocyte (α) IFN
From LDV/7 cells	25	25	<10
Control fibroblast (β)	65	<1	65
Control leukocyte (α)	6	6	<1
Control immune (γ)	25	25	25

^a The interferon titers are expressed after mixing the LDV/7 and control interferons with antiserum to fibroblast and leukocyte interferons.

TABLE III. INHIBITION OF HERPESVIRUS SAIMIRI-INDUCED CPE AND EBV EA AND TRANSFORMATION IN THE PRESENCE OF SUPERNATANT COLLECTED FROM LDV/7 CELLS, AFTER 7 DAYS OF INCUBATION

Cells/treatment/virus	Inhibition	
	In the presence of LDV/7 supernatant	In the absence of LDV/7 supernatant
1. (a) OMK cells were infected with serial dilutions of HVS with $10^{6.5}$ /ml TCID ₅₀ titer for 2 hr, cells were washed and then continuously kept at 37° in maintenance medium with and without LDV/7 supernatant for 14 days and observed for HVS-induced CPE	$10^{3.5}$ /ml	$10^{6.5}$ /ml
(b) Pretreatment of OMK cells with LDV/7 spnt. for 24 hr at 37° before HVS infection	$10^{2.5}$ to $10^{3.0}$ /ml	$10^{6.5}$ /ml
2. Raji cells were infected $10^{4.0}$ EA inducing units/ml with P3HR1 EBV for 90 min. Then the cells were washed and fed with LDV/7 supernatant for 72 hr	≤1.0% cells expressing EA and no VCA	≥10% cells expressing EA and 3% VCA
3. Ag-876 cells growing at 34° were subcultured at seeding density of $\geq 1 \times 10^6$ /ml and were fed with medium with or without LDV/7 supernatant for 7 days	10–15% EA/VCA	10–15% EA/VCA
4. HCBL separated by plasmagel were pretreated with LDV/7 supernatant (containing 600 units of interferon) at 37° for 24 hr. The other set of cord cells were kept without treatment with LDV/7 supernatant. The supernatant was removed and cells were washed with medium, infected with Ag-876 EBV with a titer of $10^{6.0}$ /ml TFU, and observed for EBNA and transformation	$10^{5.0}$	$10^{6.0}$

90% EA were inhibited when cells were kept for 72 hr in supernatant collected from 7 day-incubated cells. HCBL pretreated for 24 hr with LDV/7 supernatant containing 600 U/ml of interferon were infected with various dilutions of transforming Ag-876 EBV. The data in Table III show that up to 10 transforming units of virus were inhibited, based on EBNA induction, colonies of transformed cells, and outgrowth of these cells under soft agar.

Discussion. The spontaneous production of interferon from other human lymphoblastoid cell lines has previously been reported (6, 7, 14). Pickering *et al.* (7) showed that with the exception of two B-cell lines

(8876-7 and LukII) all other cell lines (Raji, CAH, Namalwa, BM, NBRL-AG57) either produced small amounts of interferon (10–200 units) or lacked interferon production (<10). Similarly Strander *et al.* (6) showed that the majority of the cell lines they tested produced <20 units of interferon; however, cell lines such as Namalwa, Akuba, P3HR-1, and Ly-46 could be induced to produce between 200 and 600 units of interferon. Thus LDV/7 cells may be included in the group of cells which spontaneously produce high amounts of interferon. Even though Namalwa cells can be used to produce higher yields of interferon with Sendai virus or treatment with

Poly(I)·poly(C) plus DEAE-dextran in the presence or absence of butyrate (5), the amount of interferon produced is comparable to that spontaneously produced by LDV/7 or 8866-7 and LuKII cells (300–1000 units). It seems therefore that LDV/7 cells, capable of replicating high levels of transfer factor and immune RNA, could be valuable in obtaining high yields of interferon in large-scale production without the costs and the complexity of the use of an inducer. In addition LDV/7 cells may be used for cloning the interferon gene and for studies of gene expression.

Our experiments in Fig. 1 showed that TPA, PHA-P, and LPS failed to increase the levels of interferon.

The absence of increased interferon production after mitogen stimulation may be due to interferon inhibition of mitogenic responses in LDV/7 cells. Szigeti *et al.* (15) showed that human leukocyte interferon at a concentration of 100 U/ml suppressed the mitogen (PHA)-induced lymphocyte migration. They further showed that if the lymphocytes were pretreated with interferon prior to PHA activation, it significantly reduced the action of leukocyte migration inhibitory factor. Thus the lack of increase in interferon levels in the LDV/7 supernatants in the presence of PHA or LPS may be due to the fact that LDV/7 cells are already proliferating cells and cannot be stimulated by PHA or LPS treatment. However, the action of PHA on stimulation of interferon production by T lymphocytes may be different, from the response of B cells like LDV/7 as suggested by Nathan *et al.* (14).

The kinetics of spontaneous interferon production by cell cultures has not been fully elucidated. The data of Pickering *et al.* (7) indicate that culture growth was found to correlate with the levels of interferon in cell lines spontaneously producing between 300 and 1000 units. They based this finding on spontaneously produced interferon being elaborated continuously in seeded cells into fresh medium at early saturation of density and harvesting samples of medium after incubation of 1 or 2 days. The data in Fig. 1 also indicate that interferon

production in LDV/7 cells is correlated with cell growth. Levels of interferon from Days 1, 3, and 7 were increasing and the samples from Days 10 and 14 showed that a plateau of spontaneously produced interferon was reached. Since the LDV/7 cells grow very rapidly (doubling time 24 hr) the cell saturation density occurs between 6 and 7 days and immediately after the cells are in the lag phase. Thus these data further indicate that interferon was produced only during cell growth.

The test of crude interferon against two oncogenic herpesvirus of primates indicate that it is active against HVS and EBV, supporting our previous findings where interferon obtained from both leukocyte and lymphoblastoid cells was more effective than fibroblast interferon (12). Even though P3HR-1 and Ag-876 expressing EBNA and EA/VCA, and Raji cells expressing only EBNA were not affected by the treatment with the LDV/7 interferon, these virus-infected cells could be destroyed by effector cells activated by interferon (16, 17). Since the inhibition of transformation of cord blood cells by Ag-876 EBV was observed in low multiplicity of infection, perhaps more purified and higher doses of interferon may prevent transformation even with higher levels of viruses. On the other hand, the possibility of Ag-876 being less sensitive to interferon cannot be overlooked.

The effectiveness of the interferon produced by the LDV/7 cells *in vitro* against HVS and EBV provides promising support for *in vivo* trials against EBV- and HVS-induced tumors in subhuman primates.

The possibility that this interferon has properties distinct from those of virus-induced lymphoblastoid interferon should be studied. Attempts will be made to increase the levels of interferon produced, either by examining clones of LDV/7 cells or by using other stimulating agents.

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