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Epstein–Barr virus-infected B lymphoblastoid cell lines: dynamics of interferon and 2′5′-oligoadenylate synthetase activity

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Levels of 2′-5′-oligoadenylate synthetase (2′-5′OAS) activity measured in cell-free extracts of 23 Epstein–Barr virus transformed β lymphoblastoid cell lines (LCL) were measured. Enzyme activity was low during stationary or log phase growth, and rapidly rose to peak values during log phase. Peak levels of 2′-5′OAS activity were characteristic for each LCL, and were significantly higher ($P<0.05$) in lines derived from patients with infectious mononucleosis (IM) than in lines from healthy individuals. Peak 2′-5′OAS activity correlated with maximal titers of endogenous human interferon-α (HuIFN-α); ($r = 0.80$). Enzyme activity levels could be increased by treating LCLs with exogenous HuIFN-α, or decreased by neutralization of endogenous interferon with antibody to HuIFN-α. 2′-5′OAS activity always peaked during log-phase growth, even in cultures depleted of interferon by antibody and in cultures which did not produce interferon. Thus, although peak levels of 2′-5′OAS activity in a given LCL correlated with maximal interferon titers, the growth phase associated variations in enzyme activity were independent of interferon. We conclude that regulation of constitutive levels of 2′-5′OAS in LCLs is partially independent of interferon.

2′-5′-oligoadenylate; Epstein–Barr virus; interferon; lymphoblastoid cell lines

Introduction

The expanding list of cellular activities affected by interferons has generated interest in describing mechanisms by which these molecules function [31]. Several interferon-induced enzyme activities have been described which mediate inhibition of protein synthesis at the translational level [18]. Two such enzymes, 2′-5′-oligoadenylate synthetase (2′-5′OAS) and 2′-phosphodiesterase (2′PD), have been successfully studied in cell extracts and together with a ribo-endonuclease (RNAse-L) form one pathway through which interferon's antiviral and growth inhibitory activities may be mediated [3,12,21,22,26]. Briefly, 2′-5′OAS, when activated by double-stranded RNA,

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catalyzes production of a series of oligonucleotides (collectively 2'-5'A) which have been characterized as short polymers of ATP with the structure pppA(2'p5'A)$_n$ [10]. Single stranded RNA is degraded by RNAse-L following activation of this enzyme by 2'-5'A [5,8]. 2'-5'A oligomers are degraded to AMP and ATP in a stepwise fashion by 2'PD, thus providing a mechanism for regulation of this pathway [12,28].

Epstein–Barr virus (EBV) transformed B lymphoblastoid cell lines (LCLs) are being increasingly utilized as models to study lymphocyte function and EBV-lymphocyte interactions [24]. We have investigated the status of the 2'-5'A enzyme system in newly established LCL cultures from several sources. Our results demonstate the existence of a regular variation in 2'-5'A synthesis during the growth of LCL cultures which appears to be partially independent of interferon levels. We also present evidence for higher levels of 2'-5'A synthesis in LCLs from donors with acute EBV infections, when compared with LCLs from healthy individuals.

Materials and methods

Lymphoblastoid cell lines

Two groups of LCLs were used in this study. The first group consisted of cultures initiated in this laboratory by using the following technique. Peripheral blood lymphocytes were isolated from the blood of (1) patients with acute infectious mononucleosis (IM), (2) patients with chronic EBV infections (C-EBV) associated with an inherited immunodeficiency (X-linked lymphoproliferative syndrome), and (3) healthy adults or normal human umbilical cords. The lymphocytes were obtained by centrifuging the blood samples over Ficoll–Hypaque density gradients [2]. When required, the cells were transformed by infecting isolated lymphocytes with a partially purified preparation of EBV obtained from the B95-8 cell line as previously described [23]. Alternatively, spontaneous outgrowths of in vivo transformed lymphocytes from patients with active EBV infections were selected. All LCLs in this group were initially studied within 3 wk of their first subculture.

The second group consisted of cell lines established in other laboratories, all of which had been in culture for one or more years prior to their use in this study. These were Daudi and Raji (EBV genome containing spontaneous cell lines derived from Burkitt's lymphoma patients [13,25]), and several B95-8 EBV-transformed LCLs from healthy adults (GM 558, GM 1056 and GM 1815) which were obtained from the Human Genetic Mutant Cell Repository (Camden, New Jersey).

All cultures were maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum and gentamycin (25 mcg/ml), and were subcultured twice weekly. Aliquots from all cell lines were frozen at early passage levels in complete growth medium containing 10% DMSO (v/v) using a controlled rate cell freezer (Planer Products, Ltd., Sunbury-on-Thames, England), and then stored in liquid nitrogen.

Cell growth studies

Cells from cultures at saturation density were centrifuged at 500 × *g* and the pellets resuspended in fresh growth medium at a concentration of 0.2 to 0.3 × 10^6 cells/ml. Replicate 10-ml cultures in small plastic tissue culture flasks were prepared and incubated at 37°C with 5% CO_2. Cultures were grown without further feeding except as noted. Certain cultures received additions of 100 U/ml of leukocyte-derived HuIFN-α (spec. act. 6 × 10^6 U/mg, kindly provided by Dr. Kari Cantell, Helsinki, Finland) or 100 neutralizing U/ml of rabbit antibody to HuIFN-α (Meloy Laboratories, Springfield, Virginia). Each day the cell concentrations of single cultures were determined and the cells were collected by centrifugation at 1000 × *g* for 10 min. Cell pellets and separated supernatants were frozen (–80°C). All samples from each experiment were processed and assayed together. In some experiments, large cultures were used with the daily removal of sample aliquots followed by the complete replacement of growth medium.

Interferon assays

A standard bioassay was used, registering as an end point the 50% reduction in vesicular stomatitis virus (VSV) cytopathic effect (CPE) on monolayers of human (GM 2058, Human Genetic Mutant Cell Repository, Camden, New Jersey) and bovine (EBTr) fibroblasts [33]. Samples, untreated or after adjustment of their pH to 2.0 to inactivate inferferon-γ, were assayed simultaneously on both fibroblast cell lines, and this formed the basis for differentiating between the α, β and γ types of interferon. GM 2058 fibroblasts are sensitive to HuIFN-α, -β, and -γ while EBTr fibroblasts are sensitive to HuIFN-α only [30]. Fibroblast monolayers in 96-well microtitration plates were aged for 4 days at 32°C then overnight at 37°C to maximize their sensitivity [19]. Tissue culture supernatants from LCL cultures were centrifuged at 2000 × *g* for 15 min and the pH of the aliquots to be used for the determination of HuIFN-titers were adjusted to 2.0 for 24 h. All samples were stored at –80°C until assayed. Duplicate serial two-fold dilutions were tested for each sample. Following an 18 h incubation with the test fluids, the cell monolayers were challenged with 50 μl of VSV, diluted to produce 100% CPE of unprotected monolayers in 24 h. Following a 24 h incubation at 37°C individual wells were ranked on a 0 (no CPE) to 4+ (100% CPE) scale, based on the percentage of each monolayer affected by CPE. All assays included a laboratory standard interferon preparation which had previously been assayed against international reference standards for human leukocyte (NIH G 023 901 527) and human fibroblast (NIH G 023 902 527) interferons; 50% protection of monolayers was achieved using 0.25 and 0.3 U/ml of the leukocyte interferon standard on EBTr and GM 2058 cells; 50% protection of GM 2058 cells required 1.4 U/ml of the fibroblast interferon standard. Titers are reported in units of the appropriate international reference standard which reduced CPE by 50%.

Enzyme assays

2'-5'-Oligoadenylate synthetase activity

The method of Minks et al. for 2'-5'OAS was utilized [21]. Cell pellets from 1–5 ml of cell cultures were frozen at −80°C following complete removal of supernatants. Lysates were prepared by incubating thawed pellets on ice in 50 µl of lysing buffer (20 mM HEPES/KOH, pH 7.4, 1 mM dithiothreitol, 10 mM KCl, 1.5 mM Mg $(OAc)_2$, 10% glycerol and 0.5% NP-40 v/v) for 10 min. Lysates were centrifuged at 30000 × *g* for 5 min and the resulting supernatants were stored at −80°C following determination of protein concentration (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, California). Assays were performed on 20 µl lysate aliquots containing 40 to 100 µg protein by addition of 5 µl of a reaction mix (5× concentrated for 25 mM $Mg(OAc)_2$, 20 µg/ml poly-I:poly-C, 5 mM [^{3}H]adenosine-5'-triphosphate (1.4-1.8 mCi/mmol, New England Nuclear, Boston, Massachusetts) and other components as described [21], followed by incubation at 30°C for 1–2 h. Reactions were stopped by heating in boiling water for 3 min and oligomers were then separated from nonpolymerized ATP on 0.5-ml columns of DEAE-cellulose (Whatman, DE-52) as described [21]. Samples were counted using a liquid scintillation spectrophotometer. Control assays without cell extract or without poly-I:poly-C activator were done with each assay and routinely resulted in background counts of < 500 CPM. The total incorporation of radioactivity ranged from 0.3 to 12% of the amount added. Results were standardized by subtracting backgrounds and calculating nM ATP polymerized per mg protein in the lysate per hour incubation. Results reported as 2'-5'OAS activity refer to the net accumulation of 2'-5'A oligomers and represent the sum of both synthetic and degradative activities.

2'-Phosphodiesterase

[^{32}P]-labeled 2'-5'A was obtained by using the method of Hovanessian et al. [6]. Briefly, 2'-5'OAS from interferon-treated Daudi cells was bound to poly-I:poly-C agarose beads (P-L Biochemicals, Milwaukee, Wisconsin). An 18-h incubation in a reaction mix containing 2.5 mM [α-^{32}P]-ATP (40 mCi/mmol, New England Nuclear, Boston, Massachusetts) and other components as described [6], resulted in a 10% conversion of ATP to 2'-5'A. This was separated from nonpolymerized material on a small column of DEAE-cellulose by consecutive elutions with 0.1 M and 0.4 M NH_4HCO_3. The high-salt fraction was lyophilized and reconstituted in lysing buffer (see above). The analysis of untreated and bacterial alkaline phosphatase treated material using thin layer chromatography on PEI-cellulose with either 1 M acetic acid or 1 M LiCl revealed 87% trimers, tetramers, and pentamers with < 3% contamination by nonpolymerized ATP.

The assay for 2'-PD was based on the release of AMP, and was performed by adding 20 µl of labeled 2'-5'A (containing 3×10^4 CPM) to 20 µl of cell lysate and incubating this mixture at 30°C for 2 h. The reactions were stopped by heating to 95°C for 3 min. Samples were diluted in HEPES/KOH buffer (pH 7.4) containing 90 mM KCl, and were then passed over 0.5 ml DEAE-cellulose columns three times to remove nondegraded 2'-5'A [21]. The eluate from this procedure was combined with two additional

2-ml rinses with loading buffer, and this fluid was counted in a liquid scintillation spectrophotometer. Results were expressed as CPM released per mg lysate protein per h incubation. The amount of 2'-5'A used was approximately 1 nM/assay, and maximum degradation rates were approximately 10%/h.

Results

2'-5'OAS in LCL

We assayed 2'-5'OAS activity in single cultures of a replicate series harvested on a daily schedule. Initially, cultures of 11 EBV-transformed LCLs derived from four different sources (4 patients with infectious mononucleosis, 2 with C-EBV, 4 healthy individuals and 1 patient with Burkitt's lymphoma) were studied. The results from four representative cultures are presented in Fig. 1. Levels of 2'-5'OAS activity were found to rise and peak during log phase cell growth, returning again to low levels as cultures attained saturation density. Peak 2'-5'OAS activity usually occurred on the day prior to attainment of saturation density. Most LCLs were studied twice, and peak synthetase activities for each line varied $< 15\%$ between experiments.

In these experiments, maximal 2'-5'OAS levels were higher in cell lines derived from patients with IM or C-EBV than in those derived from healthy individuals. To confirm this we studied 12 additional LCLs in a somewhat abbreviated fashion. The cells were seeded as before, but single cultures were harvested only on days 2, 3 and 4 while they were undergoing log phase growth. 2'-5'OAS activity was determined and the highest activity for each LCL was used for comparisons. Maximal levels for both sets of experiments are presented in Fig. 2. 2'-5'OAS activity in the LCLs from IM patients attained peak levels which were, on average, 4 times greater than those in LCLs originating from healthy individuals. The average maximal 2'-5'OAS activity in LCLs from C-EBV patients was threefold higher than in LCLs from healthy controls. However, it was noted that two of the three LCLs with the highest activities in this group were from patients in an active phase of their disease. Only the difference between IM and normal LCLs proved significant ($P<0.05$) with the use of a Student's *t*-test.

Since cell lines from individuals with IM mostly grew spontaneously rather than by in vitro infection with B95-8 EBV, high peak levels of 2'-5'OAS activity also correlated with spontaneous LCL initiation. Further investigation of peak 2'-5'OAS activity in paired spontaneous and B95-8 initiated cell lines derived from the same IM or C-EBV lymphocyte preparation (Table 1) failed to reveal significant differences. Paired in vitro transformations of single cord blood lymphocyte preparations with different EBV strains also failed to differ from each other in peak 2'-5'A synthetic activities (Table 1).

The normal LCLs were heterogenous with respect to in vitro age since several long term cultures were included in this group. To control for this, 4 IM-derived LCLs were studied again after 4 or more months in culture (Fig. 3). Three of these remained unchanged with respect to peak 2'-5'OAS activity while one showed a 62% decrease in

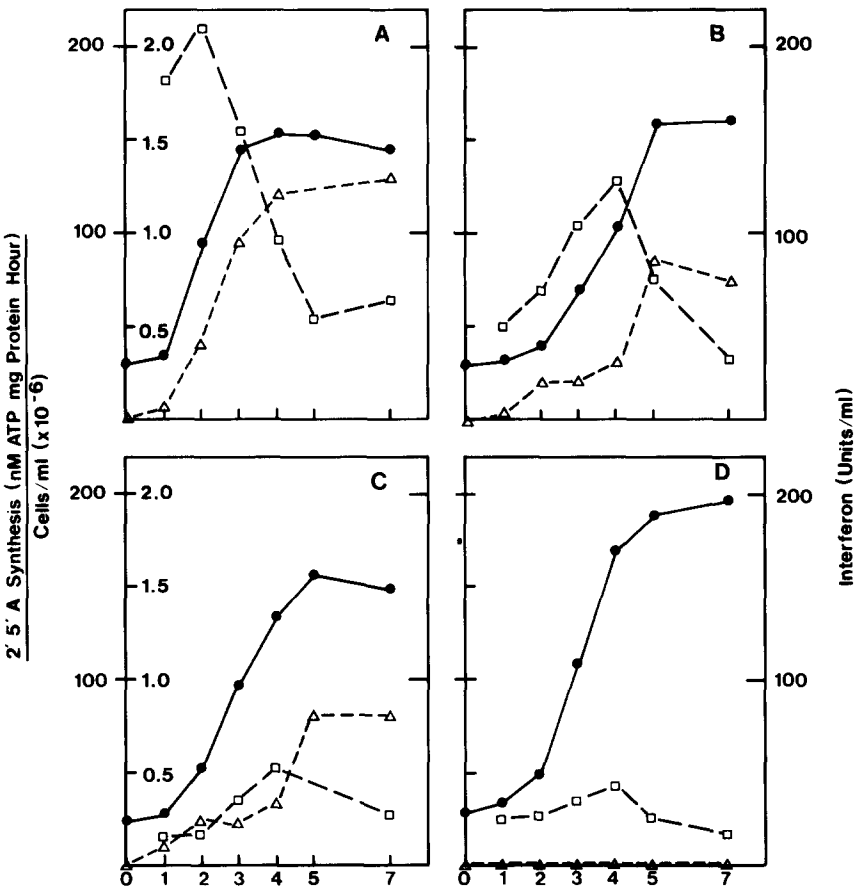

Fig. 1. Variations in 2'-5'OAS activity (□), endogenously produced interferon (△) and cell number (●) during growth of 4 EBV-transformed cell lines. Daily, single cultures of replicate series were counted, then centrifuged and cell-free extracts were prepared from pellets for 2'-5'OAS analysis. Cell lines were derived from lymphocytes of patients with IM (panel A), chronic EBV infection (panel B), Burkitt's Lymphoma (Daudi, panel D) and a healthy control (panel C).

this regard. Thus, group differences between IM-derived and normal LCLs with respect to 2'-5'A synthesis seemed to diminish with increased culture age.

Relationship between 2'-5'OAS activity and interferon in lymphoblastoid cell lines

In a first set of experiments, done on 11 LCLs, culture fluids were collected daily and interferon activity was titrated in parallel with 2'-5'OAS activity. When interferon was detected, the titers rose during log phase cell growth, then stabilized at high levels upon growth cessation (Fig. 1). The interferon detected was mainly HuIFN-α. Only very low levels ($\leq$ 4 U/ml) of HuIFN-β were observed in 3 cultures, and no HuIFN-γ

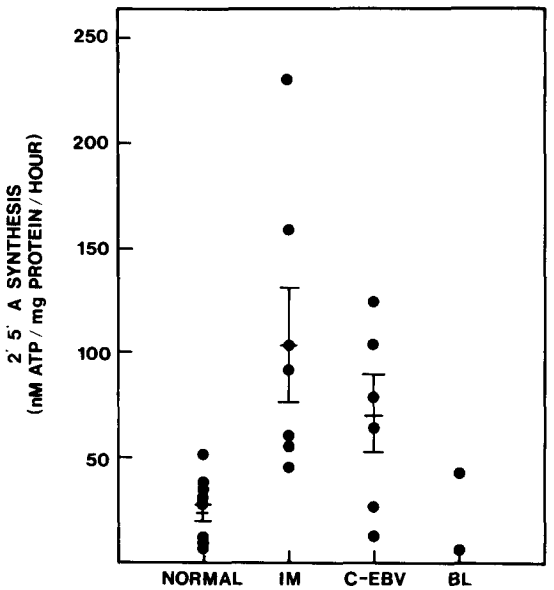

Fig. 2. Maximum values for 2'-5'OAS activity during growth of 23 EBV transformed lymphoblastoid cell lines. Lines were derived from 4 groups: healthy control (Normal), infectious mononucleosis (IM), chronic EBV infection (C-EBV) and Burkitt's Lymphoma (BL). Cell-free extracts prepared daily from single cultures of replicate series were extracted and stored at -80°C, then assayed simultaneously for each line. Peak 2'-5'OAS activities shown always occurred during the logarithmic growth phase.

TABLE 1

Peak levels[a] of 2'-5'OAS in spontaneously and EBV-transformed LCLs

Cell source[b]	Transformation			
	Spontaneous	EBV		
		B95-8	QIMR-WIL	WILD
IM	89	75	-	-
IM	49	57	-	-
C-EBV	93	107	-	-
Normal	-	27	36	-
Normal	-	59	44	-
Normal	-	29	-	35

[a] 2'-5'-Oligiadenylate synthetase activity in nM ATP/mg protein per h.

[b] Cell lines from the same lymphocyte preparation were obtained either by spontaneous outgrowth of in vivo EBV infected cells, or by in vitro infection with EBV obtained from the B95-8 or QIMR-WIL cell lines [33] or from a patient with infectious mononucleosis (WILD).

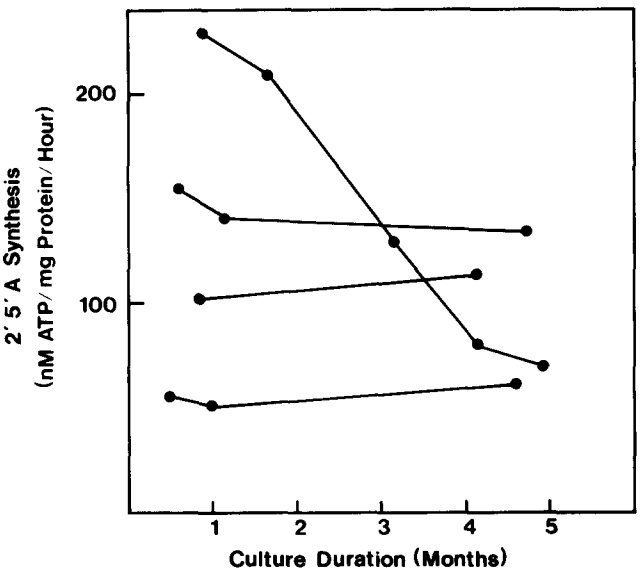

Fig. 3. 2'-5'OAS activity at different times during prolonged culture of spontaneously derived LCLs from 4 IM patients. Cultures were maintained by two weekly subculturings (1 to 4 split). Data shown are maximal values of 2'-5'OAS activity during a single subculture period. Each LCL was studied multiply during the first five months of culture, as shown.

was found. One normal LCL (GM 558) and the Burkitt's lymphoma cell line Daudi failed to produce detectable interferon.

Cultures of the other 12 LCLs were assayed for interferon when they were at saturation density. In Fig. 4 data for both sets of experiments are plotted vs. peak 2'-5'OAS activity levels. A strong correlation was found ($r = 0.80$). Also, interferon titers dropped from 128 to 86 U/ml in the IM-derived LCL which demonstrated a 62% decrease in 2'-5'OAS over a 5-mth period.

In contrast to these observations, no correlation was found when peak interferon titers were compared with 2'-5'OAS activity for samples obtained on the same day. This was due primarily to the decrease in 2'-5'OAS activity while interferon titers remained high following growth cessation. Also, 2'-5'OAS activity still peaked during log phase in two LCLs (GM 558 and Daudi) which did not produce detectable interferon (see Fig. 1 panel D).

We did further experiments in which interferon levels were artificially manipulated to more clearly define the relationship between 2'-5'OAS activity and interferon in these cell lines. In the first experiment replicate cultures of an IM-derived LCL which did not produce HuIFN-β were grown in plain growth medium or in medium supplemented with HuIFN-α (100 U/ml, added 24 h prior to harvest) or rabbit antibody to HuIFN-α (100 neutralizing U/ml added at the time of culture seeding). Single cultures were harvested daily, and samples were taken for determination of 2'-5'OAS activity and interferon titer. As shown in Fig. 5, addition of interferon caused an

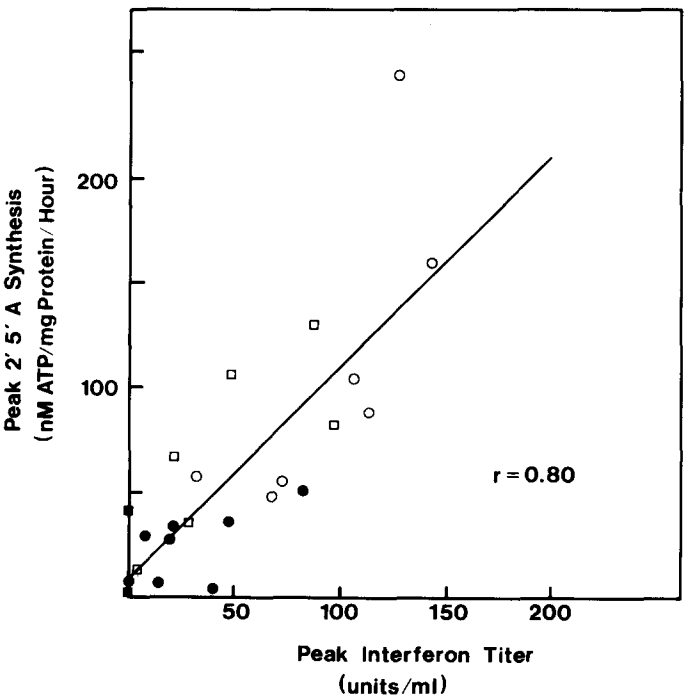

Fig. 4. Scatter plot demonstrating the relationship between highest titer of spontaneously produced interferon, and maximum 2'-5'OAS activity for each cell line studied. Each point represents one LCL as follows: healthy control (●), IM (○), C-EBV (□) and Burkitt's Lymphoma (■). Best linear fit and correlation coefficient by least squares analysis are shown.

increase in levels of 2'-5'OAS activity while neutralization of endogenous interferon caused these levels to decrease. Antiviral activity was not detectable in antibody treated cultures, while it was augmented in the supernatant of cultures to which interferon had been added. Despite the lack of detectable interferon in the fluids, antibody-treated cultures still had a clear log-phase peak in 2'-5'OAS activity which coincided with the log-phase peaks of both interferon-treated and untreated cultures. All cultures attained saturation density on day 5.

In a second experiment a single large culture of the same IM-derived LCL was studied. Samples for 2'-5'OAS activity and interferon analysis were taken daily during culture growth, and results are given in Fig. 6. Following the attainment of saturation density on day 6, a daily regimen of complete growth medium replacement was begun which initiated a second course of growth and limited the amount of supernatant interferon to the amount produced between feedings. Growth was arrested after a further 4-fold increase in cell number. A comparison of 2'-5'OAS activities for the two maintenance conditions (day 4 vs. days 8 and 9) did not reveal differences in peak levels attained. However, the peak level was prolonged concurrently with the prolongation of the log growth phase in the culture which was refed daily. Thus, log-phase peak of 2'-5'OAS activity appeared to be linked to some condition which was depen-

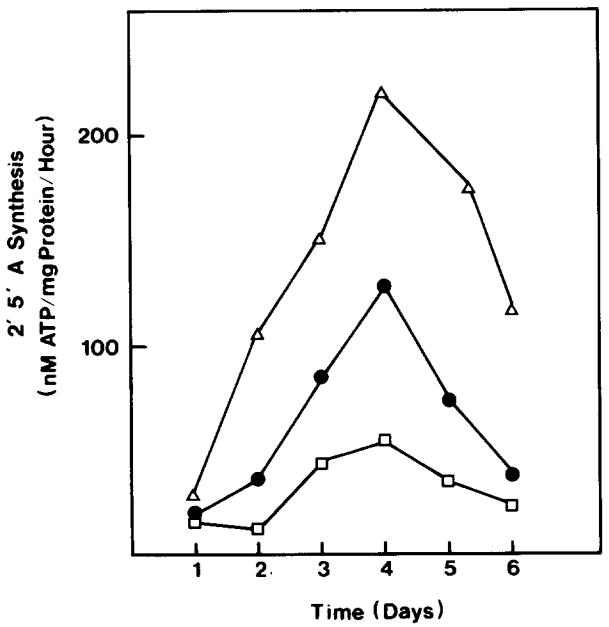

Fig. 5. Effects of addition and neutralization of interferon on 2'-5'OAS activity during growth of a single IM-derived LCL. Three treatment conditions were used as follows: 1 (△): 100 U/ml of HuIFN-α added 24 h prior to culture harvest; 2 (□): 100 U/ml of antibody to HuIFN-α added at culture seeding; 3 (●) no additions. Extracts of single cultures from each treatment condition were prepared daily, as described. 2'-5'OAS activity was measured in concurrent assays for each treatment group.

dent on logarithmic growth and independent from interferon titer.

Since accumulation of 2'-5'A in our assay system may have been influenced by 2'PD activity which degrades 2'-5'A [12,28], we did an experiment to measure the variation in this activity concurrent with determination of 2'-5'OAS activity. The experiment was conducted on the same IM-derived LCL studied above which again was grown in a large culture with daily growth medium replacement to prolong the logarithmic growth phase. The results from this experiment are presented in Fig. 7. As shown, 2'PD activity increased sharply following mid-log-phase, and high levels were maintained during the period when 2'-5'A synthesis was decreasing. These results are consistent with a role for 2'PD in the observed variation of 2'-5'A synthesis described above.

Discussion

Our results demonstrate a relationship between culture growth phase and levels of 2'-5'OAS activity in EBV-transformed LCLs. 2'-5'OAS activity increased and peaked during logarithmic cell growth, then returned to lower levels upon growth cessation in all of the 23 LCLs studied. Peak 2'-5'OAS activity levels were reproducible, and were used for making comparisons between different cell lines or different culture conditions.

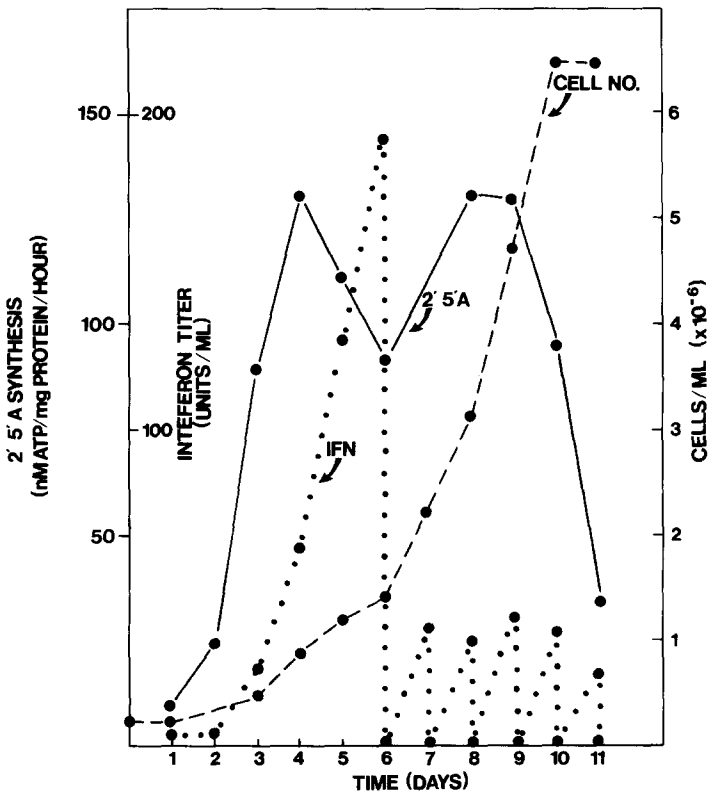

Fig. 6. Effect of different feeding regimens of 2'-5'OAS activity and spontaneous interferon production during growth of an IM-derived LCL. A single large culture was initially allowed to grow to saturation without medium replacement (days 1 to 6), then was fed daily with complete medium changes (days 6 to 11). Aliquots for cell count (---), 2'-5'A synthesis (——) and interferon titer (●●●) were obtained daily throughout the experiment.

Since 2'-5'OAS is induced by interferon, we postulated that the observed variations in 2'-5'A synthesis might be related to variations in spontaneous interferon production. In support of this postulate, a clear correlation was observed between peak levels of 2'-5'OAS activity and maximum titers of endogenously produced HuIFN-α (Fig. 4). Indeed, when interferon was added or when endogenously produced interferon was neutralized with a preparation of antibody to IFN-α, levels of 2'-5'OAS activity were raised or lowered, respectively (Fig. 5). However, these results did not explain why 2'-5'OAS activity dropped during stationary phase despite maintained high titers of endogenous interferon. In contrast to our observations other studies have reported that the highest levels of 2'-5'A synthesis occur after culture saturation [7,12,16]. These other studies were done using protocols in which either 2'-5'OAS was isolated prior to assay [7,12] or in which 2'PD activity was inhibited with high levels of ATP [16]. The latter procedures effectively eliminate degradative activities which could result in apparent losses in 2'-5'A synthesis. Our measurement of increased 2'PD

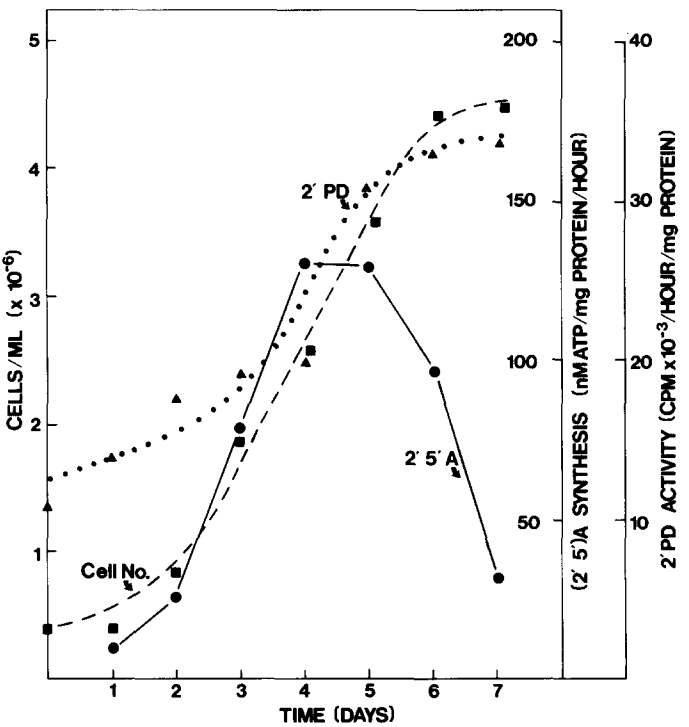

Fig. 7. Simultaneous determinations of cell number (---), 2'-5'OAS activity (——), and 2'PD activity (●●●). Cell-free extracts were prepared from aliquots removed daily from a large growing culture of an IM-derived LCL, and were assayed concurrently as described.

activity during stationary phase in one LCL (Fig. 7) suggests that 2'PD may indeed be a factor in the variation of 2'-5'A synthetic activity we describe. Further studies are needed, especially as regards correlation of results from quantitatively matched 2'-5'OAS and 2'PD assays with measurements of intracellular levels of 2'-5'A.

Our studies suggest the existence of an interferon-independent mechanism for the regulation of constitutive levels of 2'-5'OAS since peak levels during log growth phase still occurred in two LCLs which did not produce measurable interferon, and in one LCL which was grown in medium in which endogenous interferon was neutralized through use of a specific antibody. The extention of peak 2'-5'OAS activity was also noteworthy when the logarithmic growth-phase was extended by a different feeding regimen (Fig. 6), and the coincidence of log-phase 2'-5'OAS peaks despite experimental manipulation of interferon titers (Fig. 5). Several other studies support this contention. 2'-5'OAS activity has been shown to be sensitive to changes in hormones such as glucocorticoids [15] and estrogen [29] thus suggesting the existence of other mechanisms of control. Also, at least one cell line (HEC-1) maintains high levels of 2'-5'A synthesis despite a marked insensitivity to interferon [34]. Finally, Namalva cells, an LCL derived from a Burkitt's lymphoma patients, which spontaneously

produces small amounts of interferon, has been observed to undergo variations in 2'-5'OAS activity during growth which are analogous to our observations on the Daudi LCL [16].

Increased levels of 2'-5'OAS activity have been correlated with the growth-inhibitory effects of interferon [11,12,14,15,29]. We did no experiments to determine sensitivities of the cell lines to growth inhibition by 2'-5'A. However, it must be noted that the majority of cell lines grew well despite considerable titers of endogenously produced interferon and high log-phase levels of 2'-5'OAS activity. Indeed, vigorous growth was observed in freshly initiated IM-derived LCLs which also possessed the highest peak levels of interferon and 2'-5'A synthesis. In contrast, Daudi, which is known for its sensitivity to growth inhibition by interferon [1,14] produces no detectable interferon. 2'-5'OAS activity in Daudi cells is also relatively low, but can be induced to very high levels using low titers of interferon. Taken together, these observations would suggest that the initial establishment of LCLs selects for cells which are rendered insensitive to interferon's growth inhibitory effects or which produce no interferon. In support of the first postulate, Creasey et al. [4] have produced interferon-resistant clones in a human melanoma cell line by culturing cells in the presence of interferon. If 2'-5'A is involved in the growth inhibition by interferon, then it is likely that in interferon producing LCLs either 2'-5'OAS exists in a non-activated form, or that some subsequent step, such as the activation of riboendonuclease L by 2'-5'A, does not occur.

We also observed a correlation between acute EBV infections in donors and high-peak levels of 2'-5'A synthetic activity in LCLs from these individuals (Fig. 4). This phenomenon appears to be related to spontaneous production of IFN as emphasized by the case of an LCL which lost 2'-5'A synthetic activity in parallel with a loss in spontaneous interferon production during extended culture. Peripheral blood lymphocytes from patients with acute viral diseases, including IM, contain high levels of 2'-5'OAS when compared with lymphocytes from healthy adults [27]. The correlation of this phenomenon with high levels of 2'-5'A synthesis in extracts of LCLs derived from IM patients is under further investigation in our laboratory. Since interferon is an inhibitor of EBV-induced B lymphocyte transformation [17,32] further studies of the relationship between the EBV cycle, cell growth cycle, interferon and interferon-induced enzymes may lead to a better understanding of the mechanism controlling cell growth and viral transformation.

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