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Core (2′-5′)oligoadenylate and the cordycepin analog: Inhibitors of Epstein–Barr virus-induced transformation of human lymphocytes in the absence of interferon

(3′-deoxyadenosine/human leukocyte interferon/human fibroblast interferon)

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ABSTRACT **The 3′-deoxyadenosine (cordycepin) analog of (2′-5′)oligo(A) [(2′-5′)oligoadenylate with a triphosphate at the 5′ end], synthesized enzymatically from cordycepin 5′-triphosphate in lysed rabbit reticulocytes or L-cell extracts was (*i*) inhibitory to translation in lysed rabbit reticulocytes and (*ii*) metabolically stable in extracts of either L cells or C85-5C lymphoblasts. The 5′ dephosphorylated (core) (2′-5′)oligo(A) and the core cordycepin analog can replace human fibroblast interferon in preventing the transformation of human lymphocytes after infection with Epstein–Barr virus B95-8 (EBV) as determined by the decreased incorporation of [^{3}H]thymidine into cellular DNA and the inhibition of morphological transformation of EBV-infected lymphocytes. Whereas the naturally occurring core (2′-5′)oligo(A) was cytotoxic to uninfected lymphocytes and proliferating lymphoblasts, the core cordycepin analog was not. Human leukocyte interferon was more effective than human fibroblast interferon in the inhibition of EBV-induced transformation of human umbilical cord lymphocytes and adult peripheral blood lymphocytes.**

Primary exposure to Epstein–Barr virus (EBV) in childhood usually results in a silent infection (1). If infection occurs in adolescence, infectious mononucleosis develops in approximately 40% of the individuals (2). EBV infection has also been associated with Burkitt's lymphoma and nasopharyngeal carcinoma (3–5).

In vitro systems have been used to evaluate the ability of compounds to inhibit herpesvirus replication (6–13). The goal of using nucleoside analogs as antiviral agents is to develop compounds with specificity against viral-infected mammalian cells with minimum cytotoxicity. Recently, interest has grown in the possible use of interferon as an antiviral and antineoplastic agent. The development and use of interferon has been limited owing to scarcity and delivery to target cells. There have been conflicting reports on the formation of antibodies in patients receiving interferon (14, 15). Cellular transformation by oncogenic viruses has been inhibited by exogenous interferon (15–17). The effects of interferon on EBV replication in human lymphocytes have been reported (18, 19).

Interferon-treated cells induce production of cellular enzymes that affect viral replication (20–27). The inhibition of translation also involves the induction of (2′-5′)$(A)_n$ synthetase [ATP:(2′-5′)oligo(A) adenylyltransferase, EC 2.7.7.19], activated by double-stranded RNA, which polymerizes ATP into a series of (2′-5′)oligo adenylates with a triphosphate at the 5′ end, referred to as (2′-5′)oligo(A), which activate an endonuclease. The 3′-deoxyadenosine (cordycepin) analog of (2′-5′)oligo(A) has been enzymatically synthesized from rabbit reticulocyte lysates (28). Cordycepin 5′-triphosphate (3′-dATP) was selected as a substrate for (2′-5′)$(A)_n$ synthetase based on a report from this laboratory on the formation of a (2′-5′)phosphodiester bond after incorporation of 3′-dAMP into the RNA of H. Ep. 1 cells (29). Several reports exist concerning the effects of exogenous (2′-5′)oligo(A) on intact cells (30–32). The respective 5′-dephosphorylated oligonucleotide (core) can be taken up by concanavalin A-stimulated lymphocytes (33). The experiments described here show that (2′-5′)oligo(A) prevents the transformation of EBV-infected lymphocytes. A preliminary report of these results has appeared (34).

MATERIALS AND METHODS

Cell Culture and Virus Preparation. Lymphocytes were obtained from heparinized whole venous blood from the umbilical cords of newborn infants (HUCLs) or EBV-seronegative volunteers (peripheral blood lymphocytes; PBLs) from Temple University Hospital. Mononuclear leukocytes were prepared and cultured as described (35, 36). BJAB and Raji lymphoblasts were gifts of W. and G. Henle (Children's Hospital, University of Pennsylvania) and were cultured (36). C85-5C lymphoblasts were obtained by transformation of HUCLs after infection with EBV and maintained (36). EBV stocks produced from the cotton-top marmoset line B95-8, obtained from G. Miller (Yale University), had a half-maximal transforming dose (TD_{50}) per 0.20 ml of $\geq 10^4$ as determined on HUCLs (36).

Preparation of (2′-5′)Oligonucleotides. (2′-5′)oligo(A) and the core (2′-5′)oligo(A) trimer and tetramer (core trimer and core tetramer) were obtained from P-L Biochemicals. The chemical synthesis of core cordycepin analog has been described (37). The enzymatic synthesis of the cordycepin analog in lysed rabbit reticulocytes, proof of structure, inhibition of translation, and metabolic stability in HeLa cell extracts has been reported (28). L-cell extracts were prepared as described

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Abbreviations: EBV, Epstein–Barr virus; cordycepin, 3′-deoxyadenosine; 3′-dATP, cordycepin 5′-triphosphate; (2′-5′)oligo(A), oligomer of adenylic acid with (2′-5′)phosphodiester linkages and a triphosphate at the 5′-end; cordycepin analog, oligomer of 3′-deoxyadenylic acid with (2′-5′)phosphodiester linkages and a triphosphate at the 5′-end; core, 5′-dephosphorylated oligonucleotide; core trimer and tetramer, core (2′-5′)oligo(A) trimer and tetramer; HUCL, human umbilical cord lymphocytes; PBL, peripheral blood lymphocytes.

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(38). The cordycepin analog was synthesized and isolated by a modification of the synthesis of (2'-5')oligo(A) (39).

Metabolic Stability of (2'-5')Oligonucleotides. The metabolic stability of (2'-5')oligo(A) and the cordycepin analog was determined in extracts of L cells and C85-5C lymphoblasts. L-cell extracts were prepared as described (40). HUCLs and C85-5C lymphoblast extracts were prepared as described for Daudi and Raji lymphoblasts (41). (2'-5')[^{32}P]Oligonucleotides (380 nM or 5 μM) were incubated with L-cell extracts, and aliquots were removed at 0, 10, 30, and 60 min. (2'-5')[^{32}P]-Oligonucleotides (550 nM or 3.8 μM) were incubated with C85-5C lymphoblast extracts, and aliquots were removed at 0, 10, and 30 min. The products of degradation were determined by either DEAE-cellulose chromatography (42) or cellulose thin-layer chromatography in isobutyric acid/concentrated ammonia/water (66:1:33, vol/vol).

Assays for EBV-Induced Transformation. Virus stimulation of host cell DNA synthesis was measured by the [^{3}H]thymidine pulse method (43) modified for an automated cell harvester (Bellco Glass). [^{3}H]Thymidine incorporation was assayed in replicate 0.20-ml samples of EBV-infected and uninfected HUCLs or PBLs (1×10^6 cells per ml) in 96-well round-bottom microtiter plates. Seven days after infection with EBV and simultaneous treatment with oligonucleotides or interferon, cultures were pulsed with [^{3}H]thymidine (Amersham; 6 μCi/ml, 24 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) for 1 hr at 37°C. The [^{3}H]DNA retained on glass-fiber filters was assayed in 5 ml of ACS scintillation fluid (Amersham). The morphological transforming efficiencies of EBV-exposed lymphocytes were determined by colony formation in a transformed-centers assay (36).

Assessment of Cell Survival. Cell survival was accomplished by cell plating in microtiter plates through a modified transformed-centers assay (44). Cells were serially diluted into individual wells of a microtiter plate. Colonies that developed within 4 wk were counted with an inverted tissue culture microscope, and the initial colony-forming unit per seeded cell was calculated (45).

Mitogen Stimulation of Lymphocytes. HUCLs at 2×10^6 cells per ml were treated with interferon or oligonucleotides and incubated for 5 days, followed by washing and dispersion into microtiter plates. The cells were treated with pokeweed mitogen (GIBCO) for 48 hr and then assayed for DNA synthesis (43).

RESULTS

Enzymatic Synthesis and Characterization of the Cordycepin Analog of (2'-5')Oligo(A). The cordycepin analog of (2'-5')oligo(A) was synthesized and characterized from 3'-dATP with lysed rabbit reticulocytes (28) and extracts from L cells.

Metabolic Stability of the (2'-5')Oligonucleotides. The metabolic stability of oligo(A) and the cordycepin analog in extracts of L cells and C85-5C lymphoblasts was examined (Fig. 1). When (2'-5')[^{32}P]oligo(A) was incubated with either L-cell or C85-5C lymphoblast extracts, oligonucleotide degradation (90% and 20%, respectively) was observed after 30 min. In contrast, when the ^{32}P-labeled cordycepin analog was incubated with either L-cell or C85-5C lymphoblast extracts, there was no hydrolysis.

Effect of Interferon and Core (2'-5')Oligonucleotides on EBV-Induced DNA Synthesis. A significant differential exists between the DNA synthesis of EBV-infected and uninfected controls on day 7 after virus exposure, which allows measurement of both virus-induced (infected) cell and host (uninfected) cell DNA synthesis (8, 43). The ability of human leukocyte interferon, human fibroblast interferon, core trimer, core tetramer, and core cordycepin analog to inhibit this process was examined by [^{3}H]thymidine incorporation on day 7 after either HUCL (Table 1) or PBL (not shown) exposure to EBV. Human fibroblast interferon (250 units/ml) reduced [^{3}H]thymidine incorporation into EBV-infected cultures to 60% of that of control infected cells and increased DNA synthesis in uninfected cells (Table 1). This increase in DNA synthesis was also reported with fibroblast interferon-treated L cells and HeLa cells (46, 47). Core trimer (25–200 μM), core tetramer (25–100 μM), and core cordycepin analog (100–200 μM) inhibited EBV-induced host cell DNA synthesis. The (3'-5')oligo(A) core trimer did not affect DNA synthesis (not shown). The pronounced inhibition of host cell DNA synthesis observed in uninfected HUCLs and PBLs treated with core trimer (100–200 μM) and tetramer (50–100 μM) suggested that the naturally occurring (2'-5')oligonucleotides, but not the cordycepin analog, may be cytotoxic at high concentrations (Table 1).

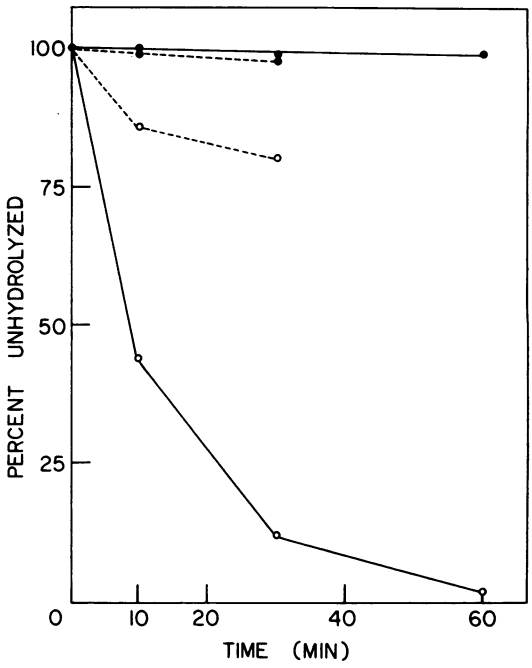

FIG. 1. Stability of (2'-5')oligo(A) and the cordycepin analog in L cell and C85-5C extracts. ^{32}P-Labeled oligonucleotides were used. Degradation of 380 nM labeled (2'-5')oligo(A) (○——○) and cordycepin analog (●——●) was determined in L-cell extracts (3.1 mg of protein per ml); degradation of 550 nM labeled (2'-5')oligo(A) (○- - -○) and cordycepin analog (●- - -●) was determined in C85-5C lymphoblast extracts (3.5 mg of protein per ml). Degradation was determined by two methods. Method one involved DEAE-cellulose chromatography as described (41). Degradation was calculated as the decrease of radioactive material eluted with 0.35 M KCl compared to the zero time point (100% undegraded). In method two, 550 nM labeled (2'-5')oligo(A) and cordycepin analog were incubated in L-cell extracts (3.1 mg of protein per ml) and 380 nM labeled (2'-5')oligo(A) and cordycepin analog were incubated with C85-5C extracts (7.5 mg of protein per ml). Degradation was determined by cellulose thin-layer chromatography.

Effect of Interferon and Core (2'-5')Oligonucleotides on EBV-Induced Morphological Transformation. The transformed-centers assay was used as a method independent of [^{3}H]thymidine incorporation to determine the effects of interferon and core (2'-5')oligonucleotides on EBV-induced transformation of PBLs (Table 2.). Transformed colonies were measured 4–6 wk after infection with EBV and simultaneous treatment with interferon or core (2'-5')oligonucleotides. Human leukocyte interferon (25–250 units/ml), human fibroblast interferon (100–500 units/ml), core trimer (50–300 μM), core tetramer (50–150 μM), and core cordycepin analog (75–300 μM) inhibited the appearance of transformed colonies. Trans-

Table 1. Effect of human fibroblast interferon and core (2'-5')oligonucleotides on spontaneous and EBV-induced stimulation of HUCL DNA synthesis

	[^{3}H]Thymidine incorporation†			
	Infected‡		Uninfected	
Treatment*	cpm	Ratio§	cpm	Ratio§
None (control)	17,450 ± 2780	1.00	7,390 ± 1220	1.00
Human IFN-β				
10 units/ml	15,000 ± 1850	0.86	9,090 ± 2030	1.22
100 units/ml	11,990 ± 1510	0.69	10,460 ± 5330	1.41
250 units/ml	10,420 ± 1080	0.60	16,550 ± 3390	2.23
Core trimer				
25 μM	7,950 ± 720	0.45	5,050 ± 820	0.68
50 μM	4,013 ± 299	0.23	4,411 ± 1416	0.59
200 μM	1,770 ± 712	0.10	1,870 ± 150	0.25
Core tetramer				
5 μM	10,580 ± 890	0.60	6,190 ± 1440	0.84
25 μM	6,460 ± 530	0.37	3,560 ± 160	0.48
100 μM	740 ± 1040	0.04	1,560 ± 620	0.21
Core cordycepin analog				
5 μM	17,790 ± 2110	1.02	7,420 ± 1230	1.00
100 μM	13,380 ± 1330	0.77	7,760 ± 2160	1.03
200 μM	8,150 ± 2320	0.47	11,580 ± 2620	1.56

IFN-β, fibroblast interferon.
* Interferon and core oligonucleotides were present continuously.
† cpm/0.2 ml of culture on day 7. Results are shown as mean ± SD.
‡ Stimulation index (SI) for EBV-infected cells, 2.36; multiplicity of infection, ≥1 infectious particle per cell.
§ Ratio of treated to nontreated cells.

formation was inhibited in a dose-dependent manner with respect to cell number and interferon or core (2'-5')oligonucleotide concentration. Core (3'-5')oligo(A) (300 μM) had no detectable effect. Human leukocyte interferon was more effective than human fibroblast interferon with respect to inhibition of EBV-induced morphological transformation (Table 3). HUCLs (Table 3) were more sensitive to the inhibitory effect of human leukocyte interferon than were PBLs (Table 2).

Effect of Interferon and Core (2'-5')Oligonucleotides on Lymphoblastoid Cell Survival. To eliminate the possibility that the effect on lymphocyte transformation by interferon or core (2'-5')oligonucleotides was due to cytotoxicity, the effect of these agents on colony formation in lymphoblasts was determined (Table 4). Neither interferon had any appreciable effect on colony formation. Core trimer at 150–300 μM and core tetramer at 50–150 μM were toxic to BJAB and Raji lymphoblasts. However, the core cordycepin analog was not cytotoxic to either BJAB or Raji lymphoblasts at concentrations up to 300 μM. Therefore, based on the concentrations of core (2'-5')oligonucleotides required to produce cytotoxicity in lymphoblasts, the inhibition of EBV-induced transformation by interferon, core trimer, core cordycepin analog, and to a lesser extent core tetramer was not the result of lymphocyte killing by these compounds.

Possible cytotoxicity of (2'-5')oligonucleotides was further investigated by treating HUCLs with core trimer at concentrations that inhibited both EBV-induced and spontaneous host cell DNA synthesis (Table 1). Five days after treatment with core trimer, HUCLs (approximately 80% viable) were mitogen-stimulated for 48 hr and assayed for DNA synthesis (Table 5). Control, core trimer-treated and core cordycepin analog-treated cells responded to pokeweed mitogen as measured by an increase in DNA synthesis over non-mitogen-treated controls. These results indicate that treatment of HUCLs with core trimer (100 μM) and core cordycepin analog (25 μM) did not result in significant cell death.

Table 2. Effect of interferon and core (2'-5')oligonucleotides on EBV-induced morphological transformation of PBLs

	Transformed-centers assay †				
Treatment*	200	70	20	7	2
None (control)	2/2	2/2	2/2	2/2	1/2
Human IFN-α					
25 units/ml	2/2	2/2	1/2	0/2	0/2
100 units/ml	1/2	1/2	0/2	0/2	0/2
250 units/ml	0/2	0/2	0/2	0/2	0/2
Human IFN-β					
100 units/ml	2/2	2/2	1/2	0/2	0/2
250 units/ml	1/2	0/2	0/2	0/2	0/2
500 units/ml	0/2	0/2	0/2	0/2	0/2
Core trimer					
10 μM	2/2	2/2	2/2	1/2	0/2
50 μM	2/2	2/2	2/2	0/2	0/2
150 μM	2/2	1/2	0/2	0/2	0/2
300 μM	0/2	0/2	0/2	0/2	0/2
Core tetramer					
10 μM	2/2	2/2	2/2	1/2	0/2
50 μM	2/2	2/2	2/2	0/2	0/2
75 μM	2/2	2/2	0/2	0/2	0/2
150 μM	0/2	0/2	0/2	0/2	0/2
Core cordycepin analog					
10 μM	2/2	2/2	2/2	2/2	0/2
50 μM	2/2	2/2	2/2	0/2	0/2
75 μM	2/2	2/2	0/2	0/2	0/2
300 μM	2/2	1/2	0/2	0/2	0/2
Core (3'-5')oligo(A) trimer					
300 μM	2/2	2/2	2/2	2/2	2/2

IFN-α, leukocyte interferon; IFN-β, fibroblast interferon.
* Interferon and core oligonucleotides were present for 7 days.
† Cell transformants per replicate exposure at cells × 10^3 per well; determined by colony formation in replicate wells of microtiter plates 4–6 wk after EBV exposure. Lymphocytes (2 × 10^6 cells per ml) were exposed to EBV at multiplicity of infection of ≈1. Simultaneously, oligonucleotides or interferon was added. After 7 days, the cells were serially diluted into replicate wells of 96-well microtiter plates. Morphologic changes were first microscopically evident several days to several weeks after exposure to virus.

DISCUSSION

The data presented here compare the inhibition of EBV-infected human lymphocytes treated with interferon with that of EBV-infected human lymphocytes treated only with the naturally occurring core trimer, core tetramer, or core cordycepin analog. The cordycepin analog inhibits translation in reticulocyte lysates and is metabolically stable in extracts of HeLa cells, L cells, and C85-5C lymphoblasts. The ^{32}P-labeled core cordycepin analog is stable in C85-5C lymphoblast media (no degradation after 6 hr), is taken up, but is not metabolized (unpublished data). Extracted RNA from cordycepin analog-treated reticulocyte lysates has a greatly reduced template activity compared to extracted RNA from (2'-5')oligo(A) tetramer-treated reticulocyte lysates when monitored in a mRNA-dependent translation system (unpublished data).

The chemical synthesis of 3'-*O*-methylated and β,γ-methylene diphosphonate analogs of (2'-5')oligo(A) has been reported (48). Baglioni *et al.* (49) suggested that the two terminal phosphates and one or two free 3'-hydroxyl groups are required for endonuclease activity. The cordycepin analog inhibits protein synthesis as well as or better than the naturally occurring (2'-5')oligo(A) (28).

Table 3. Effect of interferon on EBV-induced transformation of HUCLs

Treatment*	Transformed-centers assay† 200	70	20	7	2
None (control)	2/2	2/2	2/2	2/2	2/2
Human IFN-α					
10 units/ml	2/2	2/2	1/2	0/2	0/2
25 units/ml	2/2	1/2	0/2	0/2	0/2
500 units/ml	0/2	0/2	0/2	0/2	0/2
Human IFN-β					
50 units/ml	2/2	2/2	2/2	2/2	1/2
250 units/ml	2/2	1/2	0/2	0/2	0/2
500 units/ml	1/2	1/2	0/2	0/2	0/2

IFN-α, leukocyte interferon; IFN-β, fibroblast interferon.
* Interferon was present for 7 days.
† Cell transformants per replicate exposure at cells $\times 10^3$ per well; determined by colony formation in microtiter plates (see Table 2, footnote†).

Although human leukocyte interferon and human fibroblast interferon are effective inhibitors of EBV-induced cellular DNA synthesis and morphological transformation in lymphocytes, the same result can be achieved with core trimer, core tetramer, and core cordycepin analog. Core (3'-5')oligo(A) is without effect (Table 2). The chemically synthesized cordycepin analog or bacterial alkaline phosphatase-digested cordycepin analog possesses antiviral properties comparable to core trimer and tetramer but differs markedly with respect to the effect on spontaneous host cell DNA synthesis (Table 1). Whereas the core trimer and tetramer inhibit uninfected host cell DNA synthesis, the core cordycepin analog is without effect. Similar results are obtained when comparing high concentrations of core trimer and tetramer to core cordycepin analog in the inhibition of colony formation with lymphoblasts (Table 4). However, lymphocytes treated with core trimer (100 μM) can be mitogen-stimulated, suggesting that lymphocyte inhibition of EBV-induced transformation by core trimer, and probably core tetramer, is not due to an irreversible anticellular effect leading to cell death.

Table 4. Cytotoxicity of interferon and core (2'-5')oligonucleotides on lymphoblastoid cell lines

Treatment	Colony formation* in cell lines, % of cells Raji	BJAB	C85-5C
None (control)	5.0	2.5	10.0
Core trimer			
75 μM	5.0	2.5	ND
150 μM	2.5	1.0	ND
300 μM	1.0	0.19	ND
Core tetramer			
50 μM	5.0	2.5	ND
75 μM	0.19	1.0	ND
150 μM	0.04	0.19	ND
300 μM	<0.002	<0.002	ND
Core cordycepin analog			
300 μM	5.0	2.5	ND
Human IFN-α			
500 units/ml	ND	ND	10.0
1000 units/ml	ND	ND	10.0
Human IFN-β			
500 units/ml	5.0	2.5	10.0
1000 units/ml	ND	ND	10.0

IFN-α, leukocyte interferon; IFN-β, fibroblast interferon.
* Core (2'-5')oligonucleotides or interferon was added to cells in tubes (13 $\times$ 100 mm) at time zero, and the cells were maintained as described. After 7 days, the cells were dispersed into microtiter plates. Colony formation was measured 4–6 wk after plating and expressed as a percentage of cells forming colonies. ND, not determined

Table 5. Effect of interferon and core (2'-5')oligonucleotides on nonstimulated and mitogen-stimulated HUCL DNA synthesis measured by [^{3}H]thymidine incorporation

Treatment*	Nonstimulated† cpm§	SI¶	PWM‡ cpm§	SI¶
None (control)	1290 ± 280	1.00	8,340 ± 930	6.4
Human IFN-α				
100 units/ml	260 ± 120	1.00	630 ± 10	2.37
Core (2'-5')oligo(A) trimer				
25 μM	1010 ± 370	1.00	8,550 ± 60	8.48
100 μM	870 ± 60	1.00	8,340 ± 1360	9.63
Core cordycepin analog				
25 μM	950 ± 40	1.00	11,290 ± 2890	11.90

* Leukocyte interferon (IFN-α) and (2'-5')oligonucleotides were added at time zero; 5 days later the cells were washed and dispersed into microtiter plates.
† The incorporation of [^{3}H]thymidine into DNA of the nonstimulated cells is lower than in Table 1 but still statistically significant.
‡ Cells were treated for 48 hr with pokeweed mitogen (PWM; 10 μg/ml).
§ cpm ± variability from mean values.
¶ Stimulation Index (SI), cpm of mitogen-treated cells/per cpm of nonstimulated cells.

The morphological transformation assays indicate that core trimer, core tetramer, and core cordycepin analog inhibit EBV-induced transformation of lymphocytes as does interferon. The observation that interferon inhibits transformation of HUCLs is in contrast to a recent report (19). Interferon, core trimer, core tetramer, and core cordycepin analog inhibit herpes simplex virus I infection of PBLs (unpublished data).

The core cordycepin analog appears to be more specific in its antiviral properties compared to core trimer and tetramer, which possess antiviral and anticellular properties. It is possible that the block in EBV replication in lymphocytes is at an early event in virus replication because EBV-induced DNA synthesis, which is inhibited by the (2'-5')oligonucleotides, occurs as an early event in infected cells (43). Subsequent experiments indicate that core trimer and tetramer inhibit EBV-induced HUCL morphologic transformation and Epstein–Barr virus nuclear antigen induction by EBV in PBLs (unpublished results). Revel and coworkers (50) showed that protein synthesis is inhibited before RNA and DNA synthesis in mitogen-stimulated mouse spleen lymphocytes treated with core trimer. It is tempting to speculate that the inhibition of transformation by core trimer, core tetramer, and core cordycepin analog might affect EBV mRNA and thereby prevent virus genome amplification, which is a necessary step in lymphocyte transformation (51).

During these experiments, it became evident that interferon was not cytotoxic to lymphoblasts (Table 4) but was cytotoxic to lymphocytes (Table 5). However, at high concentrations, the naturally occuring core oligonucleotides were cytotoxic to both lymphocytes (Table 1) and lymphoblasts (Table 4). A possible explanation for the differences observed with respect to interferon sensitivity may be that in cells with a G_0 phase [i.e., lymphocytes (52)], the transition from G_0 to G_1 is blocked by interferon as reported by Merigan and coworkers (53). The lymphoblast lines used here do not possess a G_0 component and, hence, would not be susceptible to G_0–G_1 block. The observation that both lymphoblasts and virus-infected lymphocytes are sensitive to core oligonucleotides could be explained by

blocking progression of cells from G_1 to S phase as suggested by Revel and coworkers (50).

The (2′-5′)oligonucleotides represent a class of potential antiviral compounds that may be taken up by some, but not all, mammalian cells. Core trimer, core tetramer, and core cordycepin analog may be used to either supplement or replace interferon in those cells that are permeable to the core nucleotides. The core cordycepin analog is of particular interest owing to its antiviral effect coupled with its extended metabolic stability without toxicity to cells.

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