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Inhibition of Induced L-Ornithine Decarboxylase Stimulation and Erythrodifferentiation in Friend Erythroleukemia Cells by 2′,5′-Isoadenylate Trimer Core[1]

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ABSTRACT

Friend erythroleukemia cells were induced to differentiate by hexamethylene bisacetamide, dimethyl sulfoxide, and dimethylformamide. The effect of 2′,5′-oligoadenosine (trimer core) on cell growth, on erythrodifferentiation, and on the level of L-ornithine decarboxylase was tested. The results indicated a dose response-inhibitory effect of the trimer core on erythrodifferentiation. The optimal dose of the trimer inhibited hexamethylene bisacetamide and dimethyl sulfoxide stimulation of L-ornithine decarboxylase activity, with no apparent effect on cell growth, resembling the effect of high doses of interferon on both erythrodifferentiation and L-ornithine decarboxylase reported previously.

INTRODUCTION

Interferon has been shown to exert multiple effects on normal as well as on transformed mammalian cells. Among the well-studied effects are inhibition of cell growth (16, 19, 24) and virus production (14, 15, 28), inhibition (13, 21) or stimulation (14, 15) of terminal differentiation, suppression of T- and B-cell function (4, 10, 11), activation of natural killing activity (29), and activation of all macrophage functions (3, 9, 14). On the molecular level, inhibition of protein chain initiation by interferon-induced protein kinase was first demonstrated by Farrell *et al.* (5) and recently reviewed by Baglioni (1) and also by Revel (20). Another well-documented effect of interferon is the induction of 2′,5′-oligoadenosine synthetase, resulting in accumulation of 2′,5′-adenosine oligomers (8, 26). These substances, in particular the trimer oligonucleotide, were shown to activate endonuclease which degrades RNA in the cell (2).

In a previous publication, we have shown that Me_2SO-[2] and HBMA-induced differentiation is probably triggered by early stimulation of ODC (6, 7). It was also shown that the most commonly studied inhibitors of induced differentiation such as dexamethasone (23), 12-*O*-tetradecanoylphorbol-13-acetate (22), and high doses of interferon (21) inhibit ODC stimulation induced by HBMA or Me_2SO, with no apparent effect on cell growth (6, 7). In this paper, evidence is presented that the 2′,5′-oligoadenosine (trimer core) is a potent inhibitor of both FL cell differentiation and inducer-mediated stimulation of ODC activity.

MATERIALS AND METHODS

Cells. FL cells, strain 745A (6), were used throughout this study. Stationary-phase cells were cultured at 1×10^5 cells/ml in Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 15% fetal calf serum (also from Grand Island Biological Co.). Cultivation was carried out at 37° in a humidified incubator in an atmosphere of 5% CO_2-95% air. Cell cultures were allowed to grow overnight, after which the test compounds were added. Cell number was monitored every day, and the degree of differentiation was measured on Day 3 by the method of Orkin *et al.* (18). Hemoglobin content was determined in cell lysates following measurement of absorbance of cell-free lysates at 415 nm.

Determination of ODC Activity. Aliquots of cell cultures removed at the time points indicated were washed 3 times with 0.9% NaCl solution and then lysed in hypotonic buffer containing 1 mM dithiotreitol. Cell lysates were centrifuged for 10 min at $200 \times g$ in the cold, and the supernatant was collected and kept at −70°. Samples were processed within 2 to 3 days after collection. ODC activity was measured in cell extracts using L-[^{14}C]ornithine (Radiochemical Centre, Amersham, England; 40 Ci/mmol) essentially as described previously (6).

Chemicals. Me_2SO and dimethylformamide were from Fisher Scientific Co., Fair Lawn, N. J. HMBA was prepared by acetylation of 1,6-diaminohexane. 2′,5′-Oligoisoadenosine (trimer core) was kindly given by Professor Y. Lapidot of the Hebrew University Institute of Life Sciences, Jerusalem, Israel.

RESULTS

Erythrodifferentiation of FL cells is sensitive to high doses of trimer core. Thus, as shown in Chart 1, a 250 μM concentration of the substance inhibits 80 to 90% of HMBA-, Me_2SO-, or dimethylformamide-induced differentiation presented as percentage of benzidine-positive cells. Reciprocal titration of HMBA and trimer core concentrations indicate dose-dependent inhibition at all concentrations of HMBA with a maximal inhibition at about 250 μM concentration of the trimer core (Chart 2). Inhibition of induced differentiation has no effect whatsoever on growth either in the presence or the absence of inducers (Table 1).

Measurement of ODC activity in cells treated with trimer and Me_2SO or HMBA resulted in inhibition of inducer-stimulated ODC activity to the level of control cells (Chart 3). The level of ODC in cells treated with the trimer core alone is much the same as in control cells (Chart 3).

AMP tested over a wide range of concentrations had no effect on the level of differentiation induced by the various inducers. Addition of the trimer core 10 hr after the inducer also resulted in inhibition of differentiation.[3]

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[2] The abbreviations used are: Me_2SO, dimethyl sulfoxide; HMBA, hexamethylene bisacetamide; ODC, L-ornithine decarboxylase; FL, Friend erythroleukemia.

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[3] Y. Gazitt, unpublished observation.

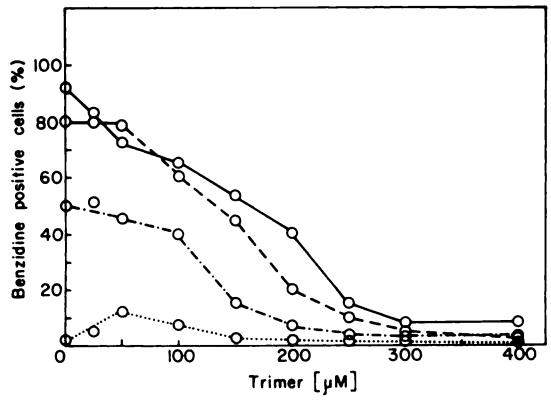

Chart 1. Effect of oligoisoadenylate (trimer core) on FL cell-induced erythrodifferentiation. ·····, trimer alone; ——, trimer plus HMBA (4 mM); – – – –, trimer plus Me_2SO (280 mM); –·–·, trimer plus dimethylformamide (220 mM). Cells were cultured and scored for benzidine-positive cells on Day 3 as described in "Materials and Methods."

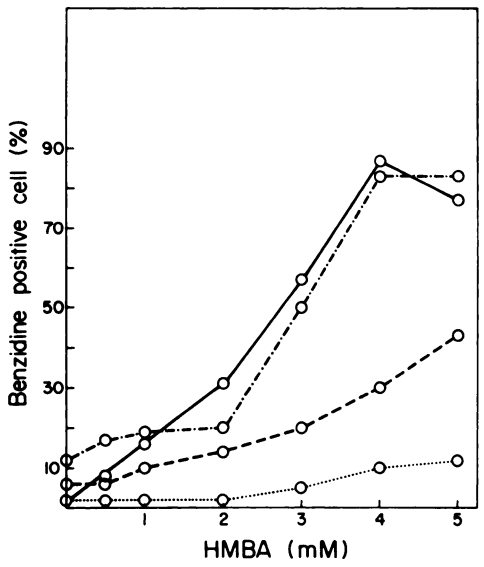

Chart 2. Dose-dependent inhibition by oligoisoadenylate (trimer core) of FL cell differentiation induced by various concentrations of HMBA. ——, HMBA alone; –·–·, HMBA plus 10 μM trimer; – – – –, HMBA plus 100 μM trimer; ·····, HMBA plus 250 μM trimer.

Table 1

Effect of oligoisoadenylate (trimer core) on growth and differentiation of FL cells

Cell cultures were prepared and the various compounds were added as described in "Materials and Methods." The cells were counted and scored for benzidine-positive cells on Day 3 of differentiation. The results are an average of 4 different experiments.

Treatment			Differentiation	
Inducer	Trimer (250 μM)	Growth (cells × 10^6/ml)	Benzidine-positive cells (% of total)	Hemoglobin[a] ($A_{415\ nm}$)
HMBA (4 mM)	–	1.8	84	0.71
Me_2SO (280 mM)	–	1.9	78	0.58
Dimethylformamide (220 mM)	–	1.7	59	0.34
	+	2.1	2	0.08
HMBA	+	1.9	18	0.15
Me_2SO	+	2.2	12	0.12
Dimethylformamide	+	1.8	7	0.11
	–	2.1	<1	0.06

[a] Cell lysates were prepared and the absorbance of hemoglobin was determined at 415 nm using a Beckman spectrophotometer. Values represent absorbance of 1 ml lysate prepared from 10^7 cells.

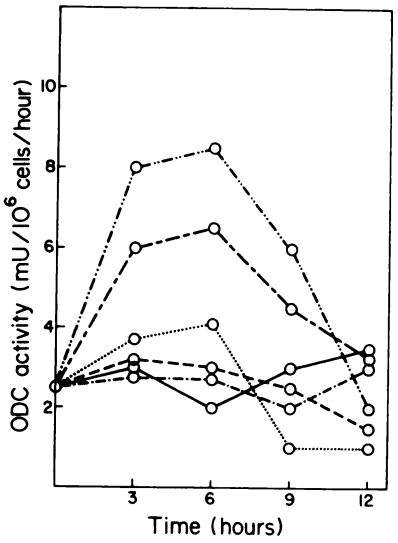

Chart 3. Effect of oligoisoadenylate (trimer core) on HMBA- and Me_2SO-induced stimulation of ODC. –·–·, no addition; ——, 250 μM trimer; ———, 4 mM HMBA; –··–, 280 mM Me_2SO; ·····, Me_2SO plus trimer; – – – –, HMBA plus trimer. Cells were cultured and inducers or trimer were added at the onset of induction as described in "Materials and Methods." Aliquots were taken for enzyme assay at the time points indicated and further processed as described in "Materials and Methods." The results represent an average of 3 different experiments carried out in duplicate samples and duplicate assays, the variation of which was less than 15%. *mU*, milliunits.

DISCUSSION

In this study, evidence is presented that 2′,5′-oligoisoadenylate trimer core inhibits Me_2SO- and HMBA-induced erythrodifferentiation of FL cells. Inhibition of differentiation is accompanied by inhibition of inducer-mediated ODC stimulation. In this respect, the effect of the trimer core resembles the effect of high doses of interferon on this system, reported previously (6, 7).

Williams and Kerr (27) have recently shown that the phosphorylated form of 2′,5′-oligoisoadenylate inhibited protein synthesis in hamster cells only following permeabilization of the cells, since the phosphorylated form of the nucleotide is not permeable to intact cells.

Kimchi *et al.* (12) have recently shown that DNA synthesis in lymphocytes stimulated to proliferate by concanavalin A could be inhibited by low concentrations of the trimer core resembling the extent of inhibition by 1000 units of interferon per ml.

These results suggest that the trimer core, which is readily permeable into the cells, can be effective in potentiating some of the interferon effects on various cell types. Although the dephosphorylated form of the trimer is more susceptible to phosphodiesterase degradation (17), the vast excess of the compound in the medium may explain its activity in spite of its possible rapid turnover.

In previous studies, we have stressed the importance of ODC in triggering differentiation of FL cells (6, 7). The fact that the trimer core inhibits ODC stimulation as early as 3 to 6 hr from its addition suggests that the activation of endonuclease by the trimer core and degradation of mRNA for ODC might be responsible for inhibition of differentiation. Thus, inhibition of globin mRNA which is evident more than 20 hr later (23) is probably a secondary effect of the inhibition of ODC synthesis. Furthermore, addition of the trimer core 10 hr after the inducer also resulted in inhibition of differentiation, suggesting that

globin mRNA might also be the target for the trimer-activated endonuclease.

This observation might also explain the finding that spermidine could not abrogate the effect of the trimer core.

Thus, inhibition of FL cell differentiation by the trimer core and possibly by interferon resemble the type of inhibition by bromodeoxyuridine in contrast to the inhibition exerted by 12-*O*-tetradecanoylphorbol-13-acetate or dexamethasone previously reported (6, 7).

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