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ANTIPROLIFERATIVE EFFECT OF (2'-5')OLIGOADENYLATE DISTINCT FROM THAT OF INTERFERON IN LYMPHOID CELLS

T. Leanderson, R. Nordfelth and E. Lundgren*

Laboratory for Cell and Tissue Culture Research,
University of Umeå, S-901 87 UMEÅ, Sweden

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SUMMARY: Interferon (IFN) induces 2'-5'oligo (A) synthetase both in P_3HR-1 cells and spleen lymphocytes. Both cell types are sensitive to the antiproliferative effect of IFN, shown by accumulation of cells in G_0/G_1. However, the reaction product of the synthetase does not mimic the effect of IFN on cell cycle parameters, rather it inhibits progression through S.

INTRODUCTION

It is not known whether the antiviral and antiproliferative effects of IFN are based upon the same chain of molecular events. One enzyme, promptly induced upon IFN addition, is 2'-5'oligo (A) synthetase (1). Its dephosphorylated reaction product 2'-5'ApApA, modulates growth of both lectin activated lymphocytes and anchorage dependent cell lines in a similar way as IFN (2,3). This effect was proposed to be mediated in fibroblasts by activation of RNase F with subsequent inhibition of protein synthesis (4) and it was proposed that the antiviral effect was achieved by the same mechanism.

The continously cycling cell line P_3HR-1 responds to IFN with growth arrest by a postmitotic accumulation in G_0 (5,6). This report shows that in lymphoid cells the 2'-5'oligo (A) system does not seem to take part in such an inhibition of proliferation.

*To whom correspondence should be addressed

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MATERIALS AND METHODS

Cell culture: The lymphoma cell line P_3HR-1 was grown in Ham's F 10 medium with 10 % newborn calf serum and antibiotics as described (5). The phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA), obtained from Consolidated Midland Corporation (Brewster, N.Y,, USA), and hydrocortisone (HC) were dissolved in ethanol (final concentration 0.1 %, also included in the controls).

Spleen cell suspensions were cultured in RPMI 1640 with 10 % fetal calf serum and supplements as described (7).

IFN preparation: Leukocyte IFN (Hu IFN-α) was produced from buffy coat lymphocytes according to standard procedures (8). Specific activity was 10^6 U/mg protein. Mouse IFN-β was purchased from LeeBiomolecular (San Diego, California, USA) with a specific activity of 1.1×10^7 U/mg protein. IFN was assayed as described (9) and its activity expressed in international reference units (U/ml) relative to the 69/19 or G002-026 standard).

Estimation of cell proliferation: Cell counts were estimated with an electronic cell counter. For flow cytofluorometry cells were stained with propidium iodide (10) and analysed as described (5). Channel of variation values (CV) were less than 5 % and each quantitation of cell cycle distribution is based on more than 50,000 cells. Thymidine incorporation was assayed as described (7).

Assay of (2'-5')oligo-isoadenylate synthetase: Cell lysis and assay was performed essentially as described by Merlin et al (11) after partial purification on poly (rI):(rC) agarose (Pl Biochemicals, Milwaukee, Wi., USA). Reaction was performed at pH 7,4 in the presence of 20 µg/ml poly (rI):(rC) (Pl Biochemicals Inc.).

After incubation alkaline phosphate digestion was performed and 2'-5'ApApA core was isolated by chromatography on DEAE-cellulose filters and counted (12).

2'-5'ApApA core (Pl Biochemicals), ApA, 3'-5'oligo (A) trimer core (Sigma, St Louis, Missouri, USA) or 2'-5'ApApA core treated with snake venom diesterase (Sigma) were dissolved in PBS. One unit of 2'-5'oligo (A) synthetase was defined as the conversion of 1pmol of ATP into 2'-5'oligo (A) per hour. Due to experimental variation, values less than 5 U were expressed as < 5 U.

RESULTS

Growth inhibitory effect of (2'-5')ApApA: In Figure 1 are compared the results obtained after addition of 2'-5'ApApA or IFN to exponentially growing P_3HR-1 cells. Panel A confirms that IFN causes the cells to enter a plateau phase after a few days of culture (5). In contrast, addition of 2'-5'ApApA only reduces the rate of growth and the cells do not enter a plateau phase.

Cell cycle distribution changes induced by (2'-5')ApApA: The postmitotic accumulation of cells in G_0 by IFN in lymphoma cell lines has previously been described by us (5,6) and others (13). To test whether this was mediated by the 2'-5'oligo (A) system, 2'-5'ApApA was added and cell cycle distribution was analysed by flow cytofluorometry.

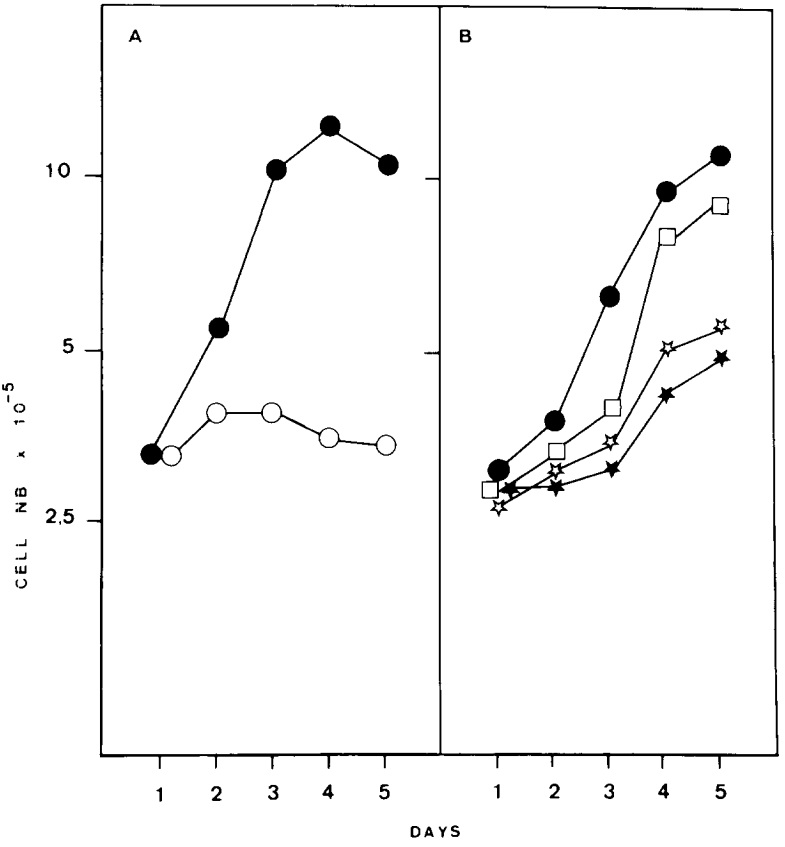

Figure 1. Effects on cell proliferation by interferon and (2'-5')ApApA as compared to controls. Panel A: Control ● , 1,000 U/ml IFN ○ . Panel B: Control ● , 2.5 μM (2'-5') ApApA □ , 5 μM (2'-5')ApApA ☆ , 10 μM (2'-5') ApApA ★ .

Figure 2 shows that IFN reduces the cycling population, increasing the G_0/G_1, i.e. resting cells, while 2'-5' ApApA increases the number of cells in the S-phase. The effect was more pronounced at the highest dose, with a decrease of cells in G_0/G_1.

Appropriate controls (table 1), including snake venom diesterase digested 2'-5'ApApA shows that the effect is specific for the oligonucleotide trimer. No significant effect could be seen after addition of 2'-5'ApA or 3'-5'ApApA.

IFN induced 2'-5'oligo (A) synthetase: Since thus a discrepancy exists in the effect of cell cycle distribution between IFN and 2'-5'ApApA treated cells, we investigated whether or not IFN could induce the 2'-5'oligo (A) synthetase in P_3HR-1 cells. Table 2 shows that this was

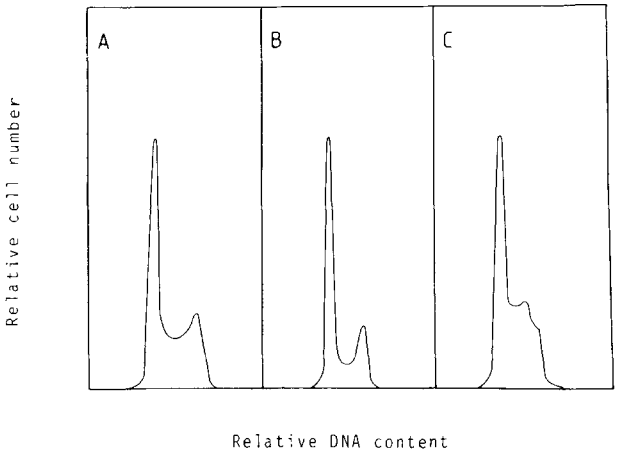

Figure 2. Cytofluorographic analysis of P_3HR-1 cells in control cells (Panel A), after addition of 1,000 U/ml IFN (Panel B) and after addition of 10 μM (2'-5')ApApA (Panel C). DNA distribution curves are based on analysis of 50,000 cells after 24 h of culture.

Table 1: Cell cycle distribution after 24 h.

Treatment	Cell cycle distribution		
	% G_0/G_1	% S	% G_2/M
Control	44	28	28
100 U/ml IFN	64	20	16
2.5 μM 2'-5'ApApA	44	33	23
5 μM ApApA	43	36	21
10 μM ApApA	38	42	20
10 μM ApA	45	32	23
10 μM 3'-5'ApApA	44	30	26
10 μM 2'-5'ApApA diesterase treated	43	30	27

Exponentially growing cells were treated with respective additive for 24 h at which time the cells were analysed in the cytofluorograph.

Table 2: Correlation between changes in cell cycle distribution and induction of 2'-5'oligo (A) synthetase.

Treatment	Cell cycle distribution			Enzymatic activity
	% G_0/G_1	% S	% G_2/M	Units/mg protein
Control	43	33	24	< 5
IFN-α (100 U/ml)	62	20	18	193
TPA (1.6×10^{-8}M)	60	26	14	< 5
Hydrocortisone (10^{-6}M)	58	29	13	28

Exponentially growing cells were treated with respective additive for 24 h before cytofluorometric analysis or 2'-5'oligo (A) synthetase activity quantitation was carried out.

Table 3: Effects of IFN and 2'-5'ApApA on cell cycle distribution and thymidine uptake in Con A activated lymphocytes.

Treatment	Cell cycle distribution			^{3}H-Tdr uptake
	% G_0/G_1	% S	% G_2/M	cpm/culture
Medium	95	3	2	1,100
Con A	64	19	17	25,000
Con A + 1,000 U/ml Mock IFN	63	20	17	25,600
Con A + 1,000 U/ml IFN	78	12	10	11,800
Con A + 10 μM 2'-5'ApApA	53	32	15	12,400

Mouse spleen cells (3×10^5/ml) were stimulated with 5 μg/ml Con A in the presence of respective additives. After 48 h triplicate cultures were either pulsed with 1 μCi/ml ^{3}H-Tdr or analyzed cytofluorographically.

the case. HC and TPA treatment was used as controls, since these compounds both cause a similar change in cell cycle distribution (table 2). HC induces some enzyme activity as reported by others (14). The level of synthetase activity does not correlate with the degree of increase of the number of G_0/G_1 cells, while TPA does not induce. Thus, the induction of enzyme activity was not due to the G_0/G_1 accumulation per se.

Con A stimulated murine lymphocytes are blocked in S: It has been previously shown that 2'-5'ApApA inhibits Con A activated murine spleen cells (2). We could confirm that IFN and 2'-5'ApApA inhibit thymidine incorporation in such cultures, but concerning cell cycle distribution there is a clear difference, since IFN causes a G_0/G_1 increase, while 2'-5'ApApA increases the S-phase cell number (table 3).

DISCUSSION

As reported (5,6) IFN causes the Burkitt's lymphoma cell line P_3HR-1 and other lymphoid cells (15) to escape out of the cell cycle into a resting G_0-like state with a dose dependent rate. This effect is in apparent contrast to those reported in other cell systems (4,16,17,18,19). The divergent data cannot, in our view, be explained solely by unpurities of IFN preparations and different assay systems used. Rather, multiple pathways for IFN action has to be postulated, causing diverse effects on cell cycle parameters in different cells.

The enzyme 2'-5'oligo (A) synthetase is induced in many cells by IFN and the reaction product has been suggested as an intracellular messenger for IFN by ribonuclease activation and subsequent hampered protein synthesis (20). Kimchi et al (4) have proposed that 3T3 cells treated with IFN and 2'-5'oligo (A) proceed from the G_0 state into the cell cycle at a reduced rate by this mechanism.

In P_3HR-1 cells, the effect of IFN was not mediated by 2'-5'oligo (A). This statement is based on the difference in kinetics and the difference in cell cycle block observed after addition of IFN and 2'-5'ApApA. Thus, concerning cell growth and cell cycle parameters, added 2'-5'oligo (A) does not mimic the effects of IFN.

By appropriate control experiments we could show that the effect was confined to the 2'-5' trimer and also applied to spleen lymphocytes. In these cells the number of cells in S is increased, while the ^{3}H-TdR incorporation is decreased. In parallell experiments we found the same in P_3HR-1 cells without effect on thymidine transport during 2'-5'oligo (A) treatment (not shown). We have reported a similar effect by IFN in a glioma cell line (19). This decreased rate of progress through S was accompanied by reduced levels of thymidine kinase and DNA polymerase (20). We propose that the postmitotic accumulation of lymphoid cells is mediated by another molecular mechanism still not known.

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REFERENCES

1. Kerr,I.M., and Brown, R.E. (1978) Proc. Natl. Acad. Sci. USA. 75, 256-260.
2. Kimchi, A., Shure, H., and Revel, M. (1979) Nature 282, 849-851.
3. Kimchi, A., Shure, H., and Revel, M. (1981) Eur. J. Biochem. 114, 5-10.
4. Kimchi, A., Shure, H., Lapidot, Y., Rapoport, S., Panet, A., and Revel, M. (1981) Febs Letters 134, 212-216.
5. Leanderson, T., and Lundgren, E. (1980) Exp. Cell. Res. 130, 421-426.
6. Leanderson, T., and Lundgren, E. (1982) J. Interferon Res. 2, in press.
7. Leanderson, T., Hillörn, V., Holmberg, D., Larsson, E.-L., and Lundgren, E. (1982) J. Immunol., in press.

8. Cantell, K. (1970) pp. 6-8, Karger, New York, Basel.
9. Lundgren, E., Larsson, I., Miörner, H., and Strannegård, Ö. (1979) J. Gen. Virol. 42, 589-595.
10. Vindelöv, L.L. (1977) Virchows Arch. B. Cell Pathol. 24, 227-242.
11. Merlin, G., Revel, M., and Wallach, D. (1981) Analyt. Biochem. 110, 190-196.
12. Harris, M. (1975) J. Cell. Physiol. 86, 413-430.
13. Attallah, A.M., Fleisher, T., Khalil, R., Noguchi, P.D., and Urritia-Shaw, A. (1980) Br. J. Cancer 42, 423-429.
14. Krishnan, I., and Baglioni, C. (1980) Proc. Natl. Acad. Sci. USA, 77, 6506-6510.
15. Roos, G., Leanderson, T., and Lundgren, E. (1982) In preparation.
16. Killander, D., Lindahl, P., Leary, O., and Gresser, I. (1976) Eur. J. Immunol. 6, 56-69.
17. Balkwill, F., and Taylor-Papadimitriou, J. (1978) Nature 274, 798-800.
18. Creasey, A.A., Bartholomew, J.C., and Merigan, T.C. (1980) Proc. Natl. Acad. Sci. USA, 77, 1471-1485.
19. Lundblad, D., and Lundgren, E. (1981) Int. J. Cancer 27, 749-754.
20. Lundblad, D., and Lundgren, E. (1982) Biochem. Biophys. Res. Commun. 105, 1569-1576.