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Effects of IFN-β on Growth of Human Prostatic JCA-1 Cells

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SUMMARY. Addition of IFN-β resulted in a dose-dependent reduction of growth, a drop in [^{3}H]thymidine incorporation into DNA, and a concurrent 69% and 15% increase in the S and G_2/M phases, of the human prostatic JCA-1 cells. No correlation existed between the antimitogenicity of IFN and increases in the double-stranded RNA-dependent protein kinase activity. Although IFN elicited a large increase in 2-5A synthetase activity, activation of the 2-5A-dependent RNase L could not be demonstrated in JCA-1 cells rendered permeable to 2-5A, implying that the 2-5A pathway is not involved in the anti-proliferative effects of IFN. Analysis of endogenous proteins phosphorylated *in vitro* show that some IFN-inducible phosphoproteins were dependent upon the presence of double-stranded DNA.

Although prostate cancer is recognized as the most common malignancy in men, there are only limited approaches to controlling its increasing mortality (1,2). This is due in part to insufficient information on the cellular and genetic characteristics of prostate carcinoma. Advances have also been hampered by the number of human prostatic cancer cell lines currently available. Moreover, the well studied PC-3, Du-145, and LNCaP cells came from metastatic sites of prostate cancer patients and were established after hormone therapy had been initiated (3-5). A recently described prostate JCA-1

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cells, on the other hand, did not have these shortcomings since they are derived from the primary site prior to the administration of hormones (6,7).

Proliferation of prostatic cells *in vitro* has been shown to be differentially suppressed by IFN although details for the reported growth modulation remain unknown (8-10). The present communication describes some of the IFN-elicited cellular and biochemical changes in JCA-1 cells.

MATERIALS AND METHODS

Cell Culture. JCA-1 cells were seeded in 6-well plates at 1 x 10^5 cells/ml ($2x10^5$/well), supplemented with 100 or 1000 IU/ml of IFN-β_{ser} (Cetus), in RPMI 1640 medium with 10% FBS, as described (6-7). Cells were harvested after 24 or 72 h and parameters described below were examined.

Effect of IFN Treatment on JCA-1 Cell Growth. Cell numbers were determined by trypan blue exclusion. To assess [^{3}H]thymidine incorporation into DNA, cells were labeled for 4-6 h with 1.6 μCi [^{3}H]thymidine/well and precipitated with trichloroacetic acid as described (11). To measure the effect of IFN on cell cycle distribution, control and treated cells (10^6 cells/0.2 ml) were incubated with RNase for 15 min at room temperature and stained in the dark with 50 μg/ml of propidium iodide. Cell cycle distribution of each sample was analyzed using EPICS PROFILE II cytometer (12).

2-5A Synthetase Assay. The poly(rI).poly(rC)-agarose and immunoblot methods were used to assay 2-5A synthetase activity (13-16).

RNase L Assay. Endogenous activity utilized the rRNA cleavage assay in cells that were permeabilized to 10 μM exogenous 2-5A, p_3A_3 or p3 A_4, as described (17). Following an additional 17 h in culture, with or without the addition of 1000 IU/ml IFN, RNA were isolated, denatured, and analyzed by agarose gel electrophoresis. Scanning of the gel was done by using a densitometer (18). RNase L activity in lysates were also determined by degradation of ^{3}H-polyadenylated HL-60 mRNA using oligo(dT) chromatography (19).

Protein Kinase Assays. The IFN-induced dsRNA-dependent protein kinase was assayed in ribosomal fractions (20). Phosphorylation of endogenous proteins, with or without poly(dA).poly(dT), was assayed using total cell extracts (21).

RESULTS AND DISCUSSION

Effects of IFN-β on Growth of JCA-1 Cells. To test the growth inhibitory activity of IFN, JCA-1 cells were treated with 100 or 1,000 IU/ml of IFN-β as described in Materials and Methods. At 1,000 IU/ml, a 40-50% inhibition of cell growth and a 80-90% reduction in [^{3}H]thymidine incorporation were observed. The effects of IFN on the cell cycle were investigated by measuring the DNA

Table 1. Effect of IFN-β on the JCA-1 Cell Cycle Distribution

Culture Conditions	% Cells in Cell Cycle Phases		
	G_o/G_1	S	G_2/M
Control	65.4	14.6	15.8
+ IFN-β (100 IU/ml)	44.1	24.2	27.2
+ IFN-β (1000 IU/ml)	48.9	24.8	18.2

contents of randomly growing control or 72 h-IFN-treated cells by flow cytometry. In control cells, the relative phase distribution was 65.4% in G_1, 14.6% in S, and 15.8% in G_2/M. A 69% and 15% increase was observed in the fraction of IFN-treated cells in the S and G_2/M phases (Table 1), suggesting that IFN induced a partial arrest of cell cycle progression (Figure 1).

Growth Inhibition by IFN-β is not correlated with the induction of dsRNA-PK. Among the many proteins whose synthesis is induced by IFN is a protein kinase with a requirement for dsRNA. The kinase is retained on a dsRNA-affinity resin and undergoes autophosphorylation in the presence of [γ-^{32}P]ATP, allowing it to

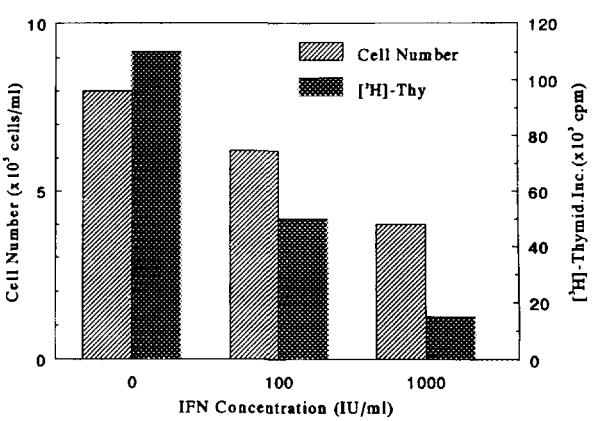


Figure 1. Effects of IFN on JCA-1 growth and [^{3}H]thymidine incorporation into DNA.

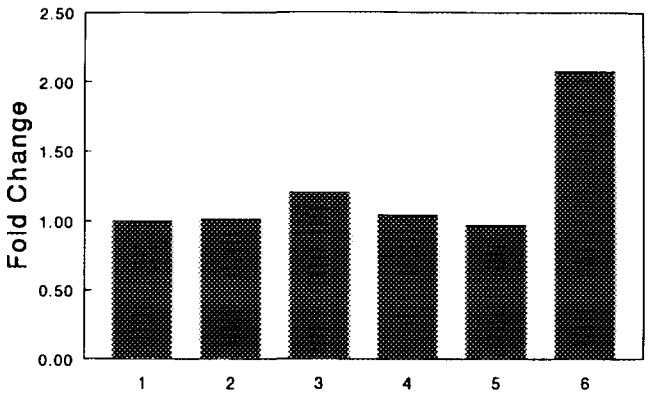
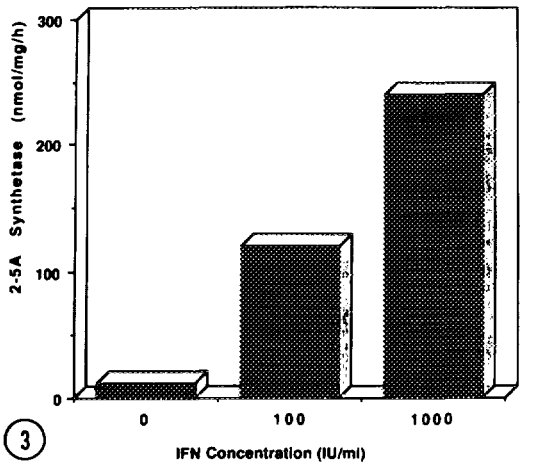


Figure 2. Induction of dsRNA-PK by IFN in JCA-1 cells. The kinase was assayed by quantitatively binding the kinase present in ribosomal salt extracts to poly(rI).poly(rC)-agarose beads as described (20). The area corresponding to the autophosphorylated p68.5 kinase was scanned and the results shown as arbitrary units. Lanes 1 and 4, control cells; lanes 2 and 5, cells treated with 100 IU/ml IFN; lanes 3 and 6, cells treated with 1000 IU/ml IFN. Lanes 1-3, assayed without the addition of dsRNA; lanes 4-6, assayed with the addition of poly(rI).poly(rC) (20 ng/ml).

be identified as a 68.5-kD entity on SDS-PAGE. To test the possible involvement of such a kinase in the anti-mitogenic property of IFN, ribosomal salt washes from control and IFN-treated cells were separately incubated with poly(rI).poly(rC)-agarose beads and the autophosphorylation of the kinase assayed. Results in Figure 2 shows that kinase is induced by the high (1000 IU/ml) but not the low (100 IU/ml) concentration of IFN. Since growth of JCA-1 cells was significantly inhibited by low dose of IFN, it seems unlikely that induction of dsRNA-PK can account for the antiproliferative effects of IFN.

Involvement of the 2-5A System in the Antiproliferative Effects of IFN-β. Another inducible protein regarded to be critical for IFN action is the enzyme 2-5A synthetase, which was induced 10- to 20-fold by IFN in JCA-1 cells (Figure 3). Since activation of the latent RNase L by 2-5A was considered to be key in the IFN-mediated arrest of cell proliferation, cells were permeabilized to 2-5A and RNase L activity assayed by rRNA degradation. RNA L-mediated rRNA cleavage products could not be detected in either



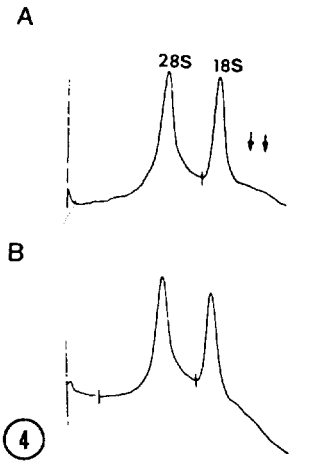


Figure 3. Induction of 2-5A synthetase by IFN in JCA-1 cells.

Figure 4. Effects of 2-5A on RNA degradation in JCA-1 cells permeabilized to 10 μM 2-5A trimer or 2-5A tetramer as described in Materials and Methods. Panel A, JCA-1 cells, 10% FBS, 10 μM 2-5A tetramer, 17 h treatment, no IFN. Panel B, JCA-1 cells, 10% FBS, 10 μM 2-5A, with IFN. The two arrows indicate the positions of migration of the RNase L-mediated-, rRNA-specific cleavage products.

control or IFN-treated cells (Figure 4), in contrast to data obtained in L-929 cells (data not shown). When nucleolytic activity was assayed by the 2-5A-enhanced degradation of [^{3}H]polyadenylated mRNA by using oligo(dT)-cellulose column chromatography, no difference could be detected between control and IFN-treated extracts (data not shown). Thus, the 2-5A pathway is also unlikely to play a major role in the IFN-elicited anti-mitogenic response.

Phosphorylation of Endogenous Proteins. Since cell cycle progression has been reported to be tightly linked to protein kinases and the overall state of phosphorylation (22-25), we examined phosphoprotein changes in control and IFN-treated cell extracts. Because IFN treatment has been shown to result in the accumulation of low molecular weight DNA (26,27), and because double-stranded DNA (dsRNA) activates a recently discovered protein kinase (DNA-PK) which has been suggested to play a key role in cell cycle control as well as a variety of other cell functions (28,29),

Table 2. Effects of poly(dA).poly(dT) on the phosphorylation of p72 in control and IFN-treated cell extracts[a]

Condition	-poly(dA).poly(dT)	+poly(dA).poly(dT)	Ratio
Control	1.0	4.77	4.77
+ IFN-β (1000 IU/ml)	2.55	4.89	1.87

[a]Five μg of cell extracts were mixed with buffer, 1.5 μCi of [γ-^{32}P]ATP (35 μM), with or without the addition of poly(dA).poly(dT) (100 ng), and incubated at 37°C for 15 min. Following SDS-PAGE, the area corresponding to p72 in the autoradiogram was scanned.

in vitro phosphorylation assays were performed in the absence or presence of dsDNA. Among the several phosphoproteins which were clearly induced by IFN (data not shown) was a protein migrating with a molecular weight of 72,000 on SDS-PAGE. The phosphorylation of p72 was found to be highly activated, and stimulated to a much lesser degree, by dsDNA, in control and IFN-treated cell extracts (Table 2). Further, its phosphorylation was dependent on the concentration of IFN added to the culture media (Figure 5). Western blot analysis show that IFN treatment did not alter the steady state level of the DNA-PK (data not shown). Thus the increase in the phosphorylation of p72 most likely reflects a change in the

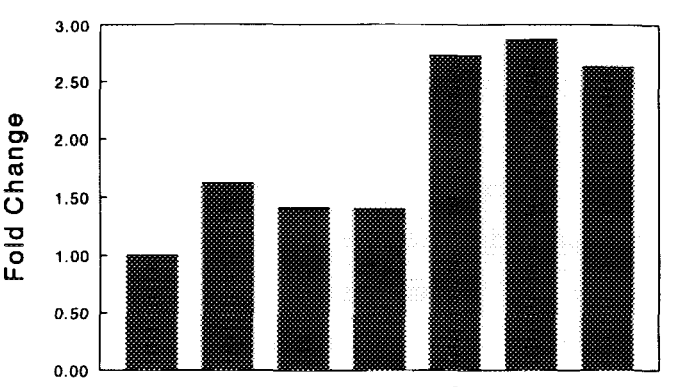


Figure 5. *In vitro* phosphorylation with control and IFN-treated cell extracts. Conditions for assays, scanning, and report of data, were as described in legend to Table 2. Lane 1, control, lanes 2-7, cells treated with 10, 25, 50, 100, 500, and 1000 IU/ml IFN.

activity of DNA-PK which could result from the IFN-elicited transient fragmentation and accumulation of DNA.

In summary, the antiproliferative effect of IFN demonstrated in JCA-1 cells is unlikely to be related to the induction of 2-5A synthetase or the dsRNA-PK by IFN. It is possible that the antimitogenicity of IFN is related to the accumulation of low molecular weight DNA and the ensued activation of DNA-PK and the concurrent phosphorylation of endogenous proteins which directly or indirectly reduces the growth of these cells.

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