Mechanism of Stimulation by Human Interferon of Prostaglandin Synthesis in Human Cell Lines

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ABSTRACT

Human interferon $\beta$ (IFN-$\beta$) stimulated the synthesis of prostaglandin E (PGE) and prostaglandin F$_{2\alpha}$ in IFN-sensitive RSa and GM258 cell lines, but not in IFN-resistant HEC-1 cells. IFN-$\beta$ at a concentration of 1000 units/ml elicited 2- to 3-fold increases in PGE production in these cell lines. In the presence of exogenous arachidonic acid (1 $\mu$g/ml), IFN-pretreated cells produced 5-fold more PGE compared to the cell cultures in the absence of arachidonic acid. Prednisolone, an inhibitor of phospholipase A$_2$, at a concentration of 2 $\mu$g/ml inhibited the enhanced synthesis of PGE by IFN-pretreated cells. Indomethacin (4 $\mu$g/ml), a potent fatty acid cyclooxygenase inhibitor, also inhibited the increased synthesis of PGE. IFN stimulated the release of $[^{14}C]$arachidonic acid from phospholipids but did not stimulate the activity of fatty acid cyclooxygenase. These data suggest that IFN stimulates prostaglandin synthesis by promoting the release of arachidonic acid from phospholipids.

Addition of exogenous PGE suppressed the growth of RSa and GM258 cells. Prednisolone and indomethacin partially inhibited anti-cell growth activity of IFN, suggesting a possibility that IFN-inhibited cell growth was partly mediated by prostaglandins.

INTRODUCTION

In addition to antiviral action, IFN$^4$ influences a variety of cell functions such as antibody formation, lymphocyte cytotoxicity, phagocytosis, cell growth, and IFN production (1, 40, 53). IFN and IFN inducers can also stimulate PG production (55), and a uniform relationship between IFN induction and cellular PG synthesis in virus-infected cell cultures was observed (9). PGs are also known to have various effects on physiological processes, and often they affect the same processes which are influenced by IFN. Therefore, PGs or other arachidonic acid metabolites may represent a potential mediator for modulating or modifying the effect of IFN. In fact, PGAs (37, 38), PGE$_2$, and PGF$_{2\alpha}$ (27, 37) inhibited the production and establishment of persistent virus infections. The addition of PGs restored the IFN response in hyporeactive animals (45). The fatty acid enzyme, cyclooxygenase, which is essential for the biosynthesis of PGs, has an important role in the establishment of the IFN-induced antiviral state (36). Mouse leukemia cells resistant to IFN are essentially devoid of fatty acid cyclooxygenase activity (4). PGE$_2$ was found to be immunosuppressive (15), and Droller et al. (7) suggested that tumor cells defend themselves from lymphocyte attack by production of PGs. Also, IFN has protected target cells from cytolysis by natural killer cells (49). Thus, some of the processes which are known to be due to IFN action might be mediated by PGs produced by cells upon IFN stimulation.

IFN-treated cells have cell surface properties different from those of nontreated cells, e.g., cell surface charge (21), mobility of cell surface ligand receptor (31, 34), cell fusion ability (46, 47), retrovirus release at cell surface (2, 10), cell surface architecture (5, 33), expression of surface antigens (26), binding of lectins (18, 24) and cholera toxin and thyrotropin (22), and exposure of cell surface gangliosides (16). Cellular phospholipase A$_2$ has been proposed to regulate membrane function by altering the levels in the membrane of lysophosphatidylcholine and free fatty acids. Recently, Chandrabose and Cuatrecasas (3) reported that the unsaturated fatty acid content of major phospholipids was decreased in IFN-treated mouse cells. This change would be expected to make membranes more rigid. Therefore, it would be interesting to determine whether the IFN-induced alterations of membrane properties correlate with the system wherein PGs are produced.

Several reports have shown that IFN stimulates the synthesis of PGs, but there is no information on the site of action of IFN in the pathway of PG synthesis. In this report, we shall present evidence that IFN acts to release the substrate arachidonic acid from phospholipids, its bound form, which in turn stimulates synthesis of PGs.

MATERIALS AND METHODS

Cells. A human fibroblast cell line, GM258, trisomic for chromosome 21, was obtained from Dr. T. Taniguchi, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan. GM258 and IF$^\beta$ cell lines were described previously (23, 25), and HEC-1 cells, resistant against IFN action, have also been described (6, 51). All cells, except GM258, were grown in monolayer culture in EMEM containing 5% FCS, streptomycin (100 $\mu$g/ml), and penicillin (100 units/ml); they were incubated at 37°C in a 5% CO$_2$ atmosphere. GM258 cells were cultivated in Roswell Park Memorial Institute Medium 1640 containing 10% FCS, plus the antibiotics noted above.

Reagents. [6-3H]Thymidine (15.0 Ci/mmol) and [2-3H]ATP (27.6 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. [4C]Arachidonic acid (55.5 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, England. Arachidonic acid and actinomycin D were purchased from Sigma Chemical Company, St. Louis, Missouri.
Mo. Authentic PGE$_2$ was kindly donated by Ono Pharmaceutical Research Laboratories, Osaka, Japan. Indomethacin was purchased from Merck Sharp & Dohme Research Laboratories, Rahway, N. J. Cycloheximide was purchased from Boehringer Mannheim GmbH, Mannheim, West Germany. Prednisolone was purchased from Takeda Pharmaceutical Institute, Ltd., Osaka, Japan. PGE and PGF$_2$ radioimmunoassay kits were purchased from Clinical Assay Inc., Cambridge, Mass. Human IFN-α (5 x 10$^6$ units/ml) and IFN-β (5 x 10$^8$ units/ml) were kindly provided by Dr. S. Yonehara, Tokyo Metropolitan Institute for Medical Research, Tokyo, Japan, and by Dr. S. Kobayashi, Basic Research Laboratories, Torey, Inc., Kamakura, Japan.

Antiviral Activity of IFN. Antiviral activity was measured by the yield reduction assay of VSV (13). Cells, cultivated in Linbro multiplates (16 mm), were treated with IFN for 20 hr. The cells were washed twice with PBS and then infected with VSV at a multiplicity of infection of 1.0. After 1 hr of virus absorption at 37°C, the cells were washed twice with EMEM, and EMEM containing 5% FCS was added to each culture. After 18 hr of incubation, the medium was harvested, centrifuged, and stored at -70°C until used for assay. The samples were assayed for VSV by the 50% tissue culture-infective dose, in microtiter plates. Antivirus Activity of IFN-β. The inhibition of cell growth was estimated by the inhibition of DNA synthesis (12, 14). Cells, cultivated in Linbro multiplates (16 mm), were treated with reagents for 48 hr. Cell monolayers were washed twice with EMEM and incubated in 0.2 ml of EMEM containing 0.1 µCi of [3H]thymidine at 37°C. After 1 hr of incubation, cells were washed twice with cold PBS and lysed by addition of 0.5 ml of 0.5% sodium sulfate dodecyl solution; then, 0.5 ml of 20% trichloroacetic acid solution was added to each plate. The radioactivities of acid-insoluble fractions were measured, as described previously (11).

2',5'-Oligoadenylate Synthetase Assay. Cell monolayers were washed with PBS, scrap off, and centrifuged for 20 min at 8000 rpm (50). Twenty µl of the cell extract were incubated for 2 hr in 25 µl of the incubation mixture containing 10 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.5), 50 mm KCl, 10 mm magnesium (acetate), 0.3 mm EDTA, 7 mm 2-mercaptoethanol, 5 mm [3H]ATP (0.8 mCi/ mmol), 10% glycerol, and polyinosinate-polycytidy late (5 µg/ml). The [3H]-2',5'-oligoadenylate synthesis was isolated by DEAE-cellulose chromatography (20, 32). The results were expressed as the total amount of radioactivity recovered as 2',5'-oligoadenylate synthetase in relation to the entire 25-µl assay.

PG Assay. At the end of the cell culture period, supernatant fluids were collected, and the levels of PGE and PGF$_2$ were measured by radioimmunoassay. When the effect of exogenous arachidonic acid on PG synthesis was tested, monolayers were washed 3 times with fresh EMEM after incubation of cells with reagents, and PBS containing arachidonic acid at the concentrations indicated was added to each plate. The cultures were incubated at 37°C, supernatant fluids were harvested at the times indicated, and levels of PGs were immediately measured by radioimmunoassay. To minimize sample-to-sample variation, the media of 3 cultures at each dose point were pooled. Fatty Acid Cyclooxygenase Assay. Cells, treated with or without reagents, were washed and suspended in 1 ml of 0.05 M phosphate buffer, pH 8.2. After sonication (5 times for 5-sec periods), cell homogenates were incubated with 0.2 µCi of [14C]arachidonic acid at 37°C for 30 min with shaking. After extraction with ethyl acetate, the PGs formed were analyzed by TLC (29) or by radioimmunoassay.

Incorporation and Release of [14C]Arachidonic Acid. Cells (5 x 10$^6$) were plated in Petri dishes (60 mm) and cultured for 24 hr; then, the medium was changed to 2 ml of EMEM containing 5% FCS and 0.2 µCi of [14C]arachidonic acid. After a labeling period of 8 hr, cell monolayers were washed 3 times with EMEM and incubated for up to 28 hr with EMEM containing 1% FCS in the presence or absence of reagents. Culture fluids (50 µl) were withdrawn, and radioactivities were determined. [14C]Arachidonic acid and [14C]PGs which were released were analyzed by TLC (29) or by radioimmunoassay. TLC plates were visualized by treatment with iodine vapor. Autoradiography was done for more precise measurement of the spots which developed.

RESULTS

Sensitivity of Cell Lines against IFN. Chart 1 shows the characteristics of cell lines used in this study. RSa cells, as reported previously (11, 12, 23, 25), were highly sensitive to anti-cell growth activity of IFN-β (Chart 1D), and GM258 cells were highly sensitive to antiviral activity of IFN-β (Chart 1C) and had moderate sensitivity to anti-cell growth activity of IFN-β (Chart 1F). HEC-1 cells were resistant to both activities of IFN-β (Chart 1, B and E). In both RSa and GM258 cells, 2',5'-oligoadenylate synthetase activity increased as a function of IFN concentrations (Chart 1, G and H), but the level of enzyme was 2-fold higher in GM258 cells. As reported previously (51), HEC-1 cells expressed a low level of enzymatic activity constitutively, but they did not respond to exogenous IFN (Chart 1H).

Stimulation of PG Synthesis by IFN. Cells were cultured in Linbro multiplates (16 mm) with various concentrations of IFN-β for specified periods, and the levels of PGE and PGF$_2$ in culture fluid were immediately measured by radioimmunoassay. Since cells in stationary phase produce only a very low level of PGs and do not respond to IFN (data not shown), semiconfluent cultures were used for the experiments. Increased accumulation of PGs in the culture media was detected at 6 hr, and their levels continued to increase linearly up to 3 days (data not shown). IFN-β-stimulated PGE and PGF$_2$ synthesis were dose dependently resistant in RSa and GM258 cells (Chart 2). IFN-β (1000 units/ml) elicited 2- to 3-fold increases in PGE production in these cell lines. IF' Cells, fairly resistant to antiviral activity of IFN, did not respond to stimulation by IFN-β; and HEC-1 cells, resistant to both antiviral and anti-cell growth activities of IFN, did not respond to the stimulation by IFN-β. Purified IFN-α also
stimulated PG production in RSa and GM258 cells (data not shown) which suggested that IFN, and not contaminants in the preparations, stimulated the production of PGs.

**Effect of Exogenous Arachidonic Acid on PG Synthesis.** The ability of cells to produce PGE was also tested by incubating cells with arachidonic acid in PBS. When arachidonic acid (1 µg/ml) was added to cell cultures for 1 hr, RSa and HEC-1 cells demonstrated enhanced production (2- to 3-fold) of PGE (Chart 3). In the presence of arachidonic acid, cells pretreated with IFN-β (1000 units/ml) for 24 hr also produced 3- to 4-fold increase of PGE compared to that in the absence of arachidonic acid. The plateau production levels of PGE were observed 10 min after addition of arachidonic acid to the RSa and HEC-1 cell cultures. However, production of PGE by IFN-pretreated GM258 cells continued to increase through 60 min, and the amount of PGE produced was 3- to 4-fold higher than that produced by RSa cells. Thus, exogenously added arachidonic acid stimulated the production of PGE and suggested that the availability of arachidonic acid appeared to limit the amount of PGE synthesis in these cells.

**Effect of Metabolic Inhibitors on PGE Synthesis.** Prednisolone, an inhibitor of phospholipase A2, at a concentration of 2 µg/ml inhibited the enhanced synthesis of PGE by IFN-pretreated GM258 cells in both the presence and the absence of exogenously added arachidonic acid (Chart 4; Table 1). Indomethacin, a potent fatty acid cyclooxygenase inhibitor, inhibited almost 90% of PGE production by both IFN-treated and nontreated cells at a concentration of 4 µg/ml. These results suggest that the increased synthesis of PGE by IFN-treated cells, both in the presence and in the absence of arachidonic acid, is dependent on phospholipase A2 and fatty acid cyclooxygenase activities. However, as shown in Table 2, IFN did not stimulate fatty acid cyclooxygenase activity, suggesting that IFN might stimulate another step of PG synthesis, the release of arachidonic acid from phospholipids. Prednisolone did not affect the activity of fatty acid cyclooxygenase in IFN-treated and in untreated cells.

Cycloheximide (5 µg/ml) and actinomycin D (0.5 µg/ml) inhibited the stimulation of PGE synthesis by IFN-β (Table 3). At these concentrations, none of the 2 reagents affected cell viability. These results suggest that the stimulation of PGE synthesis by IFN-β is due to de novo synthesis of enzymes which catalyze the synthesis of PGE.

**Release of [14C]Arachidonic Acid from IFN-treated Cells.** As shown in Table 2, IFN did not increase the activity of cyclooxygenase, therefore, we determined if increased PG synthesis could be a consequence of increased phospholipase A2 activity. GM258 cells were labeled with [14C]arachidonic acid. The cells incorporated about 80% of [14C]arachidonic acid into PGE, as indicated by radiolabeled PG E2 and F2 (Chart 3). These results suggest that IFN might stimulate another step of PG synthesis, the release of arachidonic acid from phospholipids. Prednisolone did not affect the activity of fatty acid cyclooxygenase in IFN-treated and in untreated cells.

**Table 1.** Effect of prednisolone on the conversion of exogenously added [14C]arachidonic acid into PG E2 and F2 by IFN-β-pretreated GM258 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE2 (cpm)</th>
<th>PGF2 (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1654 ± 1808</td>
<td>1748 ± 306</td>
</tr>
<tr>
<td>+ IFN-β (1000 units/ml)</td>
<td>5432 ± 634</td>
<td>4826 ± 693</td>
</tr>
<tr>
<td>+ Prednisolone (2 µg/ml)</td>
<td>330 ± 98</td>
<td>291 ± 64</td>
</tr>
<tr>
<td>+ IFN-β + prednisolone</td>
<td>333 ± 76</td>
<td>355 ± 88</td>
</tr>
</tbody>
</table>

*Cells were treated with reagents for 24 hr, washed, and incubated for 2 hr in PBS containing [14C]arachidonic acid (0.2 µCi) and cold arachidonic acid (1 µg/ml). Mean ± S.E. of 3 determinations.
medium during 8 hr incubation, and IFN did not affect the incorporation of \[^{14}C\]arachidonic acid by cells (data not shown). GM258 cells prelabeled with \[^{14}C\]arachidonic acid for 8 hr were washed and incubated with fresh medium containing 1% FCS in the presence of IFN. GM258 cells which had not been treated with IFN released 2.5, 11.9, and 13.0% of radioactive substances into culture medium in 4, 16, and 28 hr, respectively (Chart 5). Most of the radioactivity was from free arachidonic acid as identified by TLC, and a small amount of converted products, i.e., PGE\(_2\) and PGF\(_{2\alpha}\) was also detected (data not shown). IFN-treated GM258 cells released 1.6- to 1.9-fold of radioactivity compared to nontreated cells at 16 and 28 hr, respectively. Prednisolone (2 \(\mu\)g/ml) diminished the enhanced release of \[^{14}C\]arachidonic acid by IFN treatment. Indomethacin did not reverse the enhanced release of \[^{14}C\]arachidonic acid by IFN-treated cells, suggesting again that the prednisolone-sensitive step, possibly phospholipase A\(_2\) activity, is stimulated by IFN.

**Effect of PGE\(_2\) and Inhibitors of PG Synthesis on Cell Growth.** Pottathil et al. (36) reported that a fatty acid cyclooxygenase inhibitor prevented the full development of the antiviral effect of IFN. Therefore, we determined if the PGs induced by IFN were involved in the anti-cell growth activity of IFN. PGE\(_2\) at concentrations greater than 0.1 \(\mu\)g/ml inhibited cell growth in relation to dose in both RSa and GM258 cells after 48 hr of incubation (Chart 6). Addition of IFN to cultures with PGE\(_2\) suppressed cell growth additively. Prednisolone and indomethacin at concentrations above 10 \(\mu\)g/ml partially reversed the anti-cell growth activity of IFN (Table 4). When cells were treated with PGE\(_2\) at different concentrations with or without IFN-\(\beta\) (100 units/ml) for 24 hr, and DNA synthesis was measured as described in “Materials and Methods.”

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**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE(_2) (cpm)</th>
<th>PGF(_{2\alpha}) (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3350 ± 450(^a)</td>
<td>3580 ± 370</td>
</tr>
<tr>
<td>+ Prednisolone (2 (\mu)g/ml)</td>
<td>3140 ± 390</td>
<td>3250 ± 360</td>
</tr>
<tr>
<td>+ Indomethacin (4 (\mu)g/ml)</td>
<td>820 ± 140</td>
<td>910 ± 210</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone</td>
<td>3650 ± 360</td>
<td>3773 ± 520</td>
</tr>
<tr>
<td>+ IFN-(\beta) + indomethacin</td>
<td>3530 ± 230</td>
<td>3720 ± 190</td>
</tr>
<tr>
<td>+ IFN-(\beta) + cycloheximide</td>
<td>730 ± 120</td>
<td>890 ± 180</td>
</tr>
</tbody>
</table>

\(^a\) Cells were treated with reagents for 24 hr, and enzyme activity was measured as described in “Materials and Methods.”

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Production of PGE (ng/1 (\times) 10(^6) cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.28 ± 0.25(^b)</td>
</tr>
<tr>
<td>+ Cycloheximide (5 (\mu)g/ml)</td>
<td>0.93 ± 0.19</td>
</tr>
<tr>
<td>+ Actinomycin D (0.5 (\mu)g/ml)</td>
<td>0.91 ± 0.22</td>
</tr>
<tr>
<td>+ IFN-(\beta) (1000 units/ml)</td>
<td>4.01 ± 0.53</td>
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<tr>
<td>+ IFN-(\beta) + cycloheximide</td>
<td>1.01 ± 0.41</td>
</tr>
<tr>
<td>+ IFN-(\beta) + actinomycin D</td>
<td>1.17 ± 0.14</td>
</tr>
</tbody>
</table>

**Table 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA synthesis (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>+ IFN-(\beta) (100 units/ml)</td>
<td>10.3 ± 0.5(^c)</td>
</tr>
<tr>
<td>+ Prednisolone (50 (\mu)g/ml)</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone (1 (\mu)g/ml)</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone (10 (\mu)g/ml)</td>
<td>15.6 ± 0.7</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone (50 (\mu)g/ml)</td>
<td>18.3 ± 0.6</td>
</tr>
<tr>
<td>+ Indomethacin (50 (\mu)g/ml)</td>
<td>9.9 ± 0.5</td>
</tr>
<tr>
<td>+ IFN-(\beta) + indomethacin (1 (\mu)g/ml)</td>
<td>11.2 ± 0.8</td>
</tr>
<tr>
<td>+ IFN-(\beta) + indomethacin (10 (\mu)g/ml)</td>
<td>12.5 ± 0.7</td>
</tr>
<tr>
<td>+ IFN-(\beta) + indomethacin (50 (\mu)g/ml)</td>
<td>16.8 ± 0.5</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone (10 (\mu)g/ml) + indomethacin (10 (\mu)g/ml)</td>
<td>24.6 ± 6.2</td>
</tr>
<tr>
<td>+ IFN-(\beta) + prednisolone (50 (\mu)g/ml) + indomethacin (50 (\mu)g/ml)</td>
<td>33.7 ± 7.7</td>
</tr>
</tbody>
</table>

\(^a\) RSa cells were treated with reagents for 24 hr.

\(^b\) DNA synthesis was measured as in “Materials and Methods.”

\(^c\) Mean ± S.E. of 3 determinations.
treated with both reagents simultaneously, synthesis of DNA recovered to 33.7% from 10.3%, which was the level of cells which had been treated with IFN alone.

DISCUSSION

Yaron et al. (55) first reported that IFN and the IFN inducer, polyinosinate-polycytidylate, stimulated PG synthesis in cultures of synovial fibroblasts and of foreskin fibroblasts. However, Schultz et al. (43) have reported no stimulation of PG formation in macrophages. Recently, Fitzpatrick and Stringfellow (9) showed the uniform relationship between IFN induction and cellular PG synthesis in virus-infected cultures. In our experiments, IFN (1000 units/ml) elicited 2- to 3-fold increases in the production of PGE and PGF₂ by RSa, IF', and GM258 cell lines but not by IFN-resistant HEC-1 cell line (Chart 2). These data suggest that cell lines have unique sensitivities to IFN stimulation and unique enzymatic capacities for PG synthesis.

The amount of PG synthesized in RSa and GM258 cells was not high, but the levels were increased 2- to 3-fold when cells were incubated with arachidonic acid (Charts 3 and 4). This suggests that the availability of arachidonic acid seems to limit the amount of PGE synthesis in these cell lines. Since, in most tissues, fatty acids are esterified to phospholipids and triglycerides, the liberation of fatty acid from these bound stores is a necessary prerequisite for PG synthesis. Increased production of PGE by IFN-treated GM258 cells in the presence of exogenous arachidonic acid was almost completely inhibited by prednisolone treatment (Chart 4). This suggests that exogenous arachidonic acid was incorporated into phospholipids and then released by phospholipase A₂ and converted to PGE by fatty acid cyclooxygenase in GM258 cells. It is unlikely that the extracellular arachidonic acid was directly converted into PGs in IFN-treated cells, since the conversion of extracellular added [¹⁴C]arachidonic acid into [¹⁴C]PGs by IFN-treated cells was also inhibited by prednisolone (Table 1). The question is whether IFN acts to release fatty acid from phospholipids or activates the enzyme system for oxygenation and isomerization of fatty acid. Biosynthetically labeled mouse 3T3 cells with [¹⁴C]arachidonic acid contain only 1 to 3% of the radioactive arachidonic acid in the free form and the rest in the C₂ moiety of phospholipids (41, 42). If the situation is similar with mouse 3T3 cells, the release of free [¹⁴C]arachidonic acid at levels above 1 to 3% of incorporated radioactivity into GM258 cells must represent the activation of cellular phospholipase A₂. The radioactivity released from IFN-treated GM258 cells was twice that from nontreated cells (Chart 5), and it amounted to approximately 10% of incorporated radioactivity. This increased [¹⁴C]arachidonic acid release by IFN was abolished by prednisolone treatment. Furthermore, IFN did not stimulate the activity of fatty acid cyclooxygenase (Table 2). These results suggest that IFN stimulated the activity of phospholipase A₂. Since stimulation of PGE synthesis by IFN was sensitive to inhibition by cycloheximide and actinomycin D (Table 2), IFN may stimulate de novo enzyme synthesis.

There have been many contradictory reports about the action of PGs on cell growth: (a) the concentration of PGE₂ is high in hepatoma cells compared to normal hepatocytes (28, 48); (b) tumor promoter phorbol ester causes an early rise in PGE₂ (30, 52); (c) Jimenez de Asua et al. (19) reported that addition of PGE₂ to quiescent cultures of fibroblasts initiated DNA synthesis and cell proliferation. On the other hand, there are also reports that PGA (8, 17, 35) and PGE (39, 44, 54) depressed multiplication of cells in certain cell lines. In the present study, exogenously added PGE₂ suppressed the multiplication of RSa and GM258 cells (Chart 6). There is good correlation between sensitivities of cells to growth-inhibitory activity of IFN and the stimulation of PG production by these cell lines upon addition of IFN (Charts 1 and 2). Pottathil et al. (36) reported that cellular fatty acid cyclooxygenase function may be essential for the establishment of the IFN-mediated antiviral state in L-cells, since cyclooxygenase inhibitors prevented the full development of IFN protection against virus. His group also reported that a mouse cell line which was resistant to both antiviral and anti-cell growth activities of mouse IFN was essentially devoid of fatty acid cyclooxygenase activity (4). In the present study, prednisolone and indomethacin also inhibited the anti-cell growth activity of human IFN partially (Table 4). Therefore, there is a possibility that the anti-cell growth activity of IFN is at least partially mediated by PGs. Further studies are warranted before generalizing the phenomenon that the inhibition of cell growth by IFN is mediated by a mechanism involving the stimulation of PG synthesis.

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