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Inhibition of Viral Replication in Cell Cultures Treated with Prostaglandin E_1[1] (41390)

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Abstract. A study has been initiated to determine the influence of prostaglandins on virus replication and its relationship to the interferon system. In the present study, data are presented which show that pretreatment of cells in culture with prostaglandin E_1 results in (i) a reduction in the yields of Mengo, MM and polio viruses, (ii) enhanced yields of interferon, and (iii) inhibition of cell division.

Prostaglandins (PGs) appear to be involved in the interaction of viruses with host cells. However, very little work has been done to determine the effect of PGs on viral replication and/or interferon (IFN) production. Depending on the concentration used, PGE_2, $PGE_{2\alpha}$, and $PGF_{2\alpha}$ were reported to either increase or decrease the yield and spread of herpesvirus simplex (1, 2) and to suppress the replication of parainfluenza-3 virus (3) in cell cultures. PGs (A series) have recently been shown to inhibit the replication of Sendai virus in African green monkey kidney cells (4). Several PGs have been reported to restore the ability of hyporeactive animals to produce IFN in response to induction with a viral agent (5). In the present study, we report that pretreatment of cells in culture with PGE_1 results in (i) a reduction in the replication of Mengo, MM, and polio viruses, (ii) enhanced yields of IFN, (iii) inhibition of cell division.

Materials and Methods. *Cells.* L929 (L cells) and HeLa cells (S-3 clone) were grown in Dulbecco's modified MEM supplemented with 10% fetal calf serum (DMEM). Monolayer cultures for experiments were prepared by adding approximately 1.5×10^6 cells in 5 ml DMEM to 60-mm plastic dishes 18–24 hr prior to use.

Viruses. Poliovirus Brunhilde type 1 was obtained from the American Type Culture Collection, Rockville, Maryland, and was propagated and titered in HeLa cells. Stock suspensions of encephalomyocarditis virus (strains MM and Mengo) were prepared and titered by methods previously described (6). For infection of experimental cultures, virus stocks were diluted in Hanks' balanced salt solution supplemented with 2% fetal calf serum (HBSS) to give a multiplicity of infection (m.o.i.) of either 10 or 1 plaque-forming units (PFU) per cell as indicated. The virus inoculum was adsorbed for 1 hr at 25° after which each cell culture was washed twice with HBSS and 5 ml DMEM added. After 24 hr at 37°, the cells of each culture were scraped with a rubber policeman into the culture fluid. The resulting suspensions were then sonicated (Heat Systems Model W-220F; power setting 3) for 10 sec to release cell-associated virus. The cell debris was removed by low-speed centrifugation (1500*g*, 5 min) and the supernatant fluids assayed for PFU content.

Interferon assay. Twenty-four hours after infection, 1 ml of supernatant fluid was collected from each culture to be tested for IFN content. To inactivate the virus, the samples were adjusted to pH 2.0 with 1 *N* HCl for a period of 4 days at 5°. Interferon activity was determined by the 50% plaque reduction (PR_{50}) technique using MM virus as the challenge agent (7). Each PR_{50} unit by this method is equivalent to 0.5 NIH (G002-904-511) reference unit.

Prostaglandin. Prostaglandin (PG) E_1, purchased from Sigma Chemical Company, was stored as 5 mg/ml solution in 95%

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ethanol at 5° and was diluted in DMEM just prior to use.

Results. Pretreatment of cell cultures for 24 hr with PGE_1 at concentrations of 25 μg/ml and above resulted in reduced yields of Mengo (Table I), MM (Table II), and polio (Table III) viruses. Similar reductions in the amount of infectious virus produced per cell were obtained when cell cultures were treated with 10 μg PGE_1/ml for at least 48 hr (Table IV). The data in Table IV also show that prolonged treatment of HeLa and L cells with PGE_1 resulted in an inhibition of cell division. Cell viability as measured by the uptake of neutral red remained at control levels (90–95%) following treatment with all doses of PGE_1, except 100 μg/ml where it dropped to about 80% (data not shown).

Production of L cell IFN in response to MM virus infection was increased in cells pretreated for 24 hr with at least 25 μg PGE_1/ml (Table II). These data show that the final yields of IFN were dependent on both PGE_1 concentration and the multiplicity of infection. With an m.o.i. of 10, maximum IFN titers were obtained with 50 μg PGE_1/ml and remained unchanged when the hormone concentration was increased to 100 μg/ml. At an m.o.i. of 1, higher levels of IFN were produced and peaked at a PGE_1 concentration of 25 μg/ml; the IFN titers were reduced but were still above control levels at higher concentrations of the hormone.

Discussion. The data presented in this study show that pretreatment of cells in

TABLE I. EFFECT OF PRETREATMENT OF L929 CELLS WITH PGE_1 ON MENGOVIRUS REPLICATION

PGE_1 concentration (μg/ml)[a]	24-hr virus yield (PFU/ml)[b]	Percentage of control
0	1.0×10^{10}	—
10	9.5×10^{9}	95
25	5.0×10^{9}	50
50	4.5×10^{9}	45
100	7.5×10^{8}	7.5

[a] Cells in monolayer culture were treated for 24 hr with indicated concentrations of PGE_1.

[b] After the 24-hr treatment period, cell cultures were challenged with Mengovirus (10 PFU/cell). The cultures were harvested 24 hr later and the total virus (free + cell-bound) PFU titers were determined.

TABLE II. EFFECT OF PRETREATMENT OF L929 CELLS WITH PGE_1 ON MM VIRUS REPLICATION AND INTERFERON PRODUCTION

	Multiplicity of infection							
	10 PFU/cell				1 PFU/cell			
PGE_1 conc. (μg/ml)	24-hr virus yield (PFU/ml)	Percentage of control	Interferon titer (PR_{50} units/ml)	Percentage of control	24-hr virus yield (PFU/ml)	Percentage of control	Interferon titer (PR_{50} units/ml)	Percentage of control
0	8.5×10^{8}	—	3500	—	3.6×10^{8}	—	4500	—
10	6.5×10^{8}	76	3700	106	3.8×10^{8}	105	5500	122
25	5.0×10^{8}	59	2900	83	2.5×10^{8}	69	20000	444
50	4.8×10^{8}	56	8200	234	1.7×10^{8}	47	13000	288
100	2.6×10^{8}	30	8000	228	8.0×10^{7}	22	10000	222

Note. After treatment with PGE_1 at the indicated concentrations, the cell cultures were challenged with MM virus at two multiplicities of infection. Twenty-four hours later, fluids were collected and assayed for virus PFU and interferon titers.

culture with PGE_1 at a concentration of 25 μg/ml for 24 hr resulted in reduced yields of MM, Mengo, and polio viruses. Similar reductions in virus synthesis were obtained with a lower concentration of the hormone (10 μg/ml) when the cells were treated for 48 hr. Subsequent experiments indicate that PGE_1 concentrations as low as 1 μg/ml effectively inhibit virus replication when cell cultures are treated for a 4- to 8-day period (data not shown). Prolonged treatment of cell cultures with PGE_1 resulted in an inhibition of cell division (Table IV). This agrees with a similar observation reported by Thomas *et al.* (8). The reduced virus yields, however, are not merely a reflection of the reduced number of cells since the decrease in virus replication can be demonstrated on a PFU/cell basis (Table IV). The extent of antiviral activity by PGE_1 in the cells tested, is apparently dependent on the concentration of the hormone and the length of pretreatment but not on the multiplicity of infection.

The stimulation of IFN production by PGE_1 treatment could be due to a mechanism similar to that seen when cells are "primed" with IFN (9). As with most viral IFN-inducing systems, more IFN was produced at a low multiplicity of infection. There was no correlation between virus yields and the amount of IFN produced. This observation agrees with that previously reported for this virus–cell system (9).

These results are similar to those obtained when cells in culture are treated with IFN and support the notion that, in some virus–cell systems, there is a strong relationship between IFN and prostaglandins

TABLE III. EFFECT OF PGE_1 PRETREATMENT OF HeLa CELLS ON POLIOVIRUS REPLICATION

PGE_1 concentration (μg/ml)	24-hr virus yield (PFU/ml)	Percentage of control
0	9.1×10^9	—
10	7.7×10^9	85
25	5.5×10^9	60
50	3.2×10^9	35
100	5.0×10^8	6

Note. Cells in monolayer culture were treated for 24 hr with indicated concentrations of PGE_1 and then infected with poliovirus Brunhilde type 1 (10 PFU/cell).

TABLE IV. EFFECT OF PROLONGED TREATMENT OF CELL CULTURES WITH A LOW CONCENTRATION OF PGE_1 ON VIRUS REPLICATION

Length of treatment (hr) with 10 μg PGE_1/ml	No. HeLa cells/culture at time of infection	24-hr poliovirus yield (PFU/cell)	Percentage of control	No. L929 cells/culture at time of infection	24-hr Mengovirus yield (PFU/cell)	Percentage of control
0	5.0×10^6	1900	—	2.5×10^6	3900	—
24	4.5×10^6	1800	95	2.5×10^6	3500	90
48	4.0×10^6	1045	55	2.1×10^6	2400	62
72	3.2×10^6	500	26	1.8×10^6	800	20
96	2.5×10^6	380	20	1.4×10^6	300	8

Note. Tissue culture plates (60 mm) were seeded with either HeLa cells or L929 cells (1×10^6 cells/plate). PGE_1 (10 μg/ml final concentration) was added to duplicate plates of each cell line at 24-hr intervals. Four days later, cultures ranged in PGE_1 treatment from 0 to 96 hr. The cells were counted in each treatment group and all cultures were then infected with the virus indicated at an m.o.i. of 10 PFU/cell.

in the establishment of the antiviral state (10). That this is not true in every virus–cell system, is suggested by other studies showing that PGs enhance the spread of herpesvirus simplex in cell cultures (1, 2) and inhibit the production of IFN (11).

We are currently investigating the effect of PGE_1 treatment of cells in culture on the replication of DNA viruses. In addition, the influence of PGE_1 on the course of virus infection and IFN production *in vivo* is being determined.

1. Harbour DA, Blyth WA, Hill TJ. Prostaglandins enhance spread of herpes simplex virus in cell cultures. J Gen Virol **41**:87–95, 1978.
2. Newton A. Inhibitors of prostaglandin synthesis as inhibitors of herpes simplex virus replication. Advan Ophthalmol **38**:58–63, 1979.
3. Luczak M, Gumulka W, Szmigielski S, Korbecki M. Inhibition of multiplication of parainfluenza 3 virus in prostaglandin-treated WISH cells. Arch Virol **49**:377–380, 1975.
4. Santoro MG, Benedetto A, Carruba G, Garaci E, Jaffe BM. Prostaglandin A compounds as antiviral agents. Science **209**:1032–1034, 1980.
5. Stringfellow DA. Prostaglandin restoration of the interferon response of hyporeactive animals. Science **201**:376–378, 1978.
6. Giron DJ, Pindak FF. Propagation of MM virus in L cells. Appl Microbiol **17**:811–814, 1969.
7. Giron DJ, Liu RY, Hemphill FE, Pindak FF, Schmidt JP. Role of interferon in the antiviral state elicited by selected interferon inducers. Proc Soc Exp Biol Med **163**:146–150, 1980.
8. Thomas DR, Philpott GW, Jaffe BM. The relationship between concentration of PGE and rates of cell replication. Exp Cell Res **84**:40–45, 1974.
9. Giron DJ, Allen PT, Pindak FF, Schmidt JP. Interferon-inducing characteristics of MM virus. Appl Microbiol **21**:387–393, 1971.
10. Chandrabose K, Pottathil R, Cuatrecases P, Lang DJ. Role of prostaglandins in interferon mediated antiviral state. Fed Proc **39**:1–3, 1980.
11. Trofatter KF, Daniels CA. Effect of prostaglandins and cyclic adenosine 3′,5′-monophosphate modulators on herpes simplex growth and interferon response in human cells. Infect Immun **27**: 158–167, 1980.

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