Induction of Viral Resistance by Poly I:C in Cells Which Apparently Produce No Interferon[1] (36713)

DAVID J. GIRON, JEROME P. SCHMIDT, AND FRANK F. PINDAK

Epidemiology Division, USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas 78235

The synthetic double-stranded polyribonucleotide, polyinosinic–polycytidylic acid (poly I:C) is a potent viral inhibitor whose mechanism of action is presumed to be dependent upon induction of interferon. Low concentrations of poly I:C, however, have been shown to induce cellular resistance to viral infection in the absence of detectable interferon (1–3). Circumstantial evidence was recently reported which suggested that even at low inducer concentrations, interferon was a necessary intermediate for the development of the antiviral state (4, 5). Schafer and Lockart (4) showed that Vero cells, which respond to interferon but cannot produce it, did not become resistant after poly I:C treatment, whereas LLC-MK_2 cells, which both produce and respond to interferon, developed resistance after poly I:C exposure. McCoy cells, whose response to interferon is similar to that of Vero cells, were used in the present study to further investigate the relationship of interferon induction to resistance to viral infection after poly I:C treatment.

Materials and Methods. Cells, medium and viruses. McCoy cells (6) and L cells were grown in Blake glass bottles in a modified Eagle's medium (MEM) (7) containing Hanks' balanced salt solution (HBS), twice the prescribed concentration of amino acids and vitamins, and supplemented with 10% fetal calf serum (FCS). Monolayer cultures

TABLE I. Protection of McCoy and L Cells by L-Cell Interferon.

	24-hr virus yield (PFU/ml) and cytopathic effect (CPE)[b]			
	L cells		McCoy cells	
Interferon dilution[a]	Virus yield	CPE	Virus yield	CPE
1:4	2.9×10^4	0	1.1×10^4	0
1:8	4.0×10^4	0	3.4×10^4	0
1:16	2.4×10^5	0	1.1×10^5	0
1:32	3.7×10^6	0	2.3×10^6	0
1:64	5.4×10^6	0	8.1×10^6	0
1:128	5.3×10^7	0	3.2×10^7	0
1:256	8.0×10^7	0	5.2×10^7	0
1:512	1.1×10^8	2+	8.5×10^8	1+
1:1024	1.6×10^8	3+	1.1×10^8	2+
Control	2.6×10^8	4+	3.1×10^8	4+

[a] Interferon prepared in L cells was diluted in MEM + 5% FCS. Duplicate cell cultures were treated with each interferon dilution for 18 hr. The cultures were then washed and challenged with MM virus (10 PFU/cell).

[b] 0 = no CPE; 1+ = 25% CPE; 2+ = 50% CPE; 3+ = 75% CPE; 4+ = 100% CPE.

[1] The research reported in this paper was conducted by personnel of the Epidemiology Division, USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Base, TX. Further reproduction is authorized to satisfy the needs of the U.S. Government.

TABLE II. Interferon Induction by MM Virus in McCoy and L Cells.

Challenge virus[a]	48-hr interferon yield (PR_{50} units)	
	McCoy cells	L cells
MML_1	<3	8000
MML_2	<45[b]	5000
$MM(BHK_3)L_3$	<3	2000
$MM(BHK_3)L_4$	<3	2400

[a] MM virus stocks harvested from mouse brains, differ in the number of subsequent passages in BHK 21 and L cells. Subscript numbers indicate the number of passages in each cell line.

[b] Lower dilutions of this preparation were not tested.

were prepared by adding 3.5×10^6 cells in 10 ml of MEM to 100-mm plastic dishes (Falcon) or 1.5×10^6 cells in 5 ml MEM to 60-mm dishes, 18 to 24 hr before use. MM virus was harvested from mouse brains and passed in either L cells, BHK 21 cells, or both, for the number of times indicated in the text. Columbia SK virus (Col. SK) was propagated in L cells. Plaque assay of both viruses was done on L cells as previously described (8).

Production and assay of interferon. Interferon was prepared by infecting monolayer cultures of L cells with MM virus at a multiplicity of 1 plaque-forming unit (PFU)/cell. Fluids were collected 48 hr after virus inoculation and stored at −70°. Virus activity was eliminated by UV irradiation and the interfering agent was characterized as interferon (9). Assay for interferon activity was by the 50% plaque reduction (PR_{50}) technic using MM virus as the challenge agent (10). To determine the protective capacity of the interferon preparation, duplicate cell cultures (60-mm) were treated overnight with various dilutions of interferon (5 ml/plate). The cultures were then washed with HBS and challenged with MM virus (10 PFU/cell). The fluids and cells were collected 24 hr later and, after 3 cycles of rapid freezing and thawing, the clarified fluid was assayed for virus content.

Poly I:C and actinomycin D. Poly I:C was purchased from Microbiological Associates and diluted in MEM containing DEAE-dextran (100 μg/ml). In each experiment the treatment period with poly I:C was 1 hr. Actinomycin D (Cal. Biochem.) treatment was for 1 hr at a concentration of 5 μg/ml.

Results. Production and response of McCoy and L cells to interferon. Table I shows a comparison of the protective effect of exogenous L-cell interferon in McCoy and L cells. The data show that the protection against cytopathic effect (CPE) and virus replication was essentially the same in both cell lines. Table II shows that McCoy cells

TABLE III. Interferon Production and MM Virus Replication in McCoy and L Cells Treated With Poly I:C.

Poly I:C treatment[a] (μg/ml)	Interferon[b] induced by poly I:C		24-hr virus yield[c] (PFU/ml)		Interferon[d] induced by virus	
	L cells	McCoy cells	L cells	McCoy cells	L cells	McCoy cells
0	<3	<3	2.6×10^6	1.3×10^7	12,000	<3
0.1	<3	<3	4.8×10^5	8.9×10^6	3000	<3
0.5	35	<3	6.0×10^2	2.1×10^6	<3	<3
1.0	300	<3	<50	7.8×10^5	<3	<3
5.0	1500	<3	<50	2.0×10^3	<3	<3
10.0	2500	<3	<50	1.0×10^3	<3	<3

[a] Cells were treated with poly I:C (2 plates at each conc) for 1 hr.

[b] Interferon activity (PR_{50} unit) 18 hr after poly I:C treatment.

[c] Cultures were infected with MM virus (about 0.1 PFU/cell) 18 hr after poly I:C treatment.

[d] Interferon activity in virus-yield samples.

did not produce detectable amounts of interferon in response to different suspensions of MM virus. L cells, however, produced interferon of relatively high titer. These data show that McCoy cells respond to the protective action of interferon but do not produce it, whereas L cells produce and respond to interferon.

Protection by poly I:C. The protection of McCoy and L cells by various concentrations of poly I:C was determined, with MM virus as the challenge agent (Table III). The absence of interferon production by McCoy cells in response to either poly I:C or virus again demonstrates the inability of these cells to produce this substance. It is clear, however, that in spite of the absence of interferon production, poly I:C treatment protected McCoy cells against virus replication. As little as 0.5 μg/ml of the drug resulted in a significant reduction in virus yield. Higher concentrations dramatically reduced the amount of virus produced. As expected, L cells produced interferon in response to both poly I:C and virus infection and were protected by the inducer.

To determine if the protection of McCoy cells by poly I:C treatment extended to another virus, the previous experiment was repeated with Col. SK virus as the challenge agent. The effect of the drug on cell division was also determined. The results are shown in Table IV. In general, the data were similar to those depicted in Table III. Poly I:C protected both cell lines against the virus. However, it is apparent that the drug was not as effective against Col. SK virus as it was against MM virus. It is also apparent that, at the concentrations tested, poly I:C did not interfere with cell division.

Effect of actinomycin D on protection of poly I:C and interferon. Development of protection in McCoy cells after poly I:C treatment does not appear to require the synthesis of interferon. An experiment was designed to determine if an active cellular function was required for the antiviral action of poly I:C in this cell line. Cultures of McCoy and L cells were treated with actinomycin D or MEM for 1 hr. Some of the cultures were then treated with poly I:C for 1 hr, while the

TABLE IV. Effect of Poly I:C Treatment on Cell Division, Interferon Production and Columbia SK Virus Replication by McCoy and L Cells.

Poly I:C treatment	Interferon induced by poly I:C		Cells/culture[a] ($\times 10^6$)		24-hr virus yield[b] (PFU/culture)		Interferon induced by virus[c]	
(μg/ml)	L cells	McCoy cells	L cells	McCoy cells	L cells	McCoy cells	L cells	McCoy cells
0	<3	<3	3.2	3.2	5.4×10^7	6.8×10^7	7800	<3
0.1	<3	<3	2.8	4.0	6.0×10^7	4.4×10^7	2800	<3
0.5	<3	<3	3.5	3.4	5.8×10^6	4.4×10^7	90	<3
1.0	<3	<3	3.3	3.8	6.4×10^6	4.0×10^7	60	<3
5.0	1000	<3	3.0	3.5	2.2×10^2	5.6×10^5	32	<3
10.0	2000	<3	3.2	3.7	80	6.0×10^4	<3	<3

[a] Cells per culture at the time of virus infection (36–42 hr after seeding). All cultures were seeded with 1.5×10^6 cells.
[b] Cultures were inoculated with Columbia SK virus (about 0.1 PFU/cell) 18 hr after poly I:C treatment.
[c] Interferon activity in virus-yield samples.

TABLE V. Effect of Actinomycin D Treatment on Protection by Poly I:C and Interferon in L and McCoy Cells.

	24-hr virus yield (PFU/ml)			
	L cells		McCoy cells	
Treatment[a]	Actinomycin D	Control	Actinomycin D	Control
Poly I:C	4.2×10^7	7.2×10^3	1.0×10^7	1.6×10^5
Interferon	4.0×10^7	4.8×10^3	1.6×10^7	2.2×10^4
MEM	3.2×10^7	5.4×10^6	9.2×10^6	1.2×10^7

[a] Replicate cultures were treated with either poly I:C (10 μg/ml) or fresh MEM for 1 hr. One-half of the cultures were then treated with actinomycin D (5 μg/ml) for 1 hr. Some of the cultures which had been treated only with actinomycin D or MEM were then exposed to L-cell interferon (4000 PR_{50} units) for 18 hr, while the rest of the plates received MEM. All of the cultures were then challenged with MM virus at a multiplicity of about 0.1 PFU/cell.

others received interferon or MEM for 18 hr. They were then challenged with MM virus and the cells and fluids were harvested 24 hr later. The results (Table V) show that actinomycin D treatment abolished the protective effect of poly I:C and interferon in both cell lines. These data indicate that, as in L cells, the development of the antiviral state in McCoy cells after poly I:C or interferon treatment required an active cellular process.

Discussion. The notion is prevalent that the protective action of poly I:C is dependent on the production of interferon. Even at low protective concentrations of the drug, which elicit no interferon, it is assumed by some that *undetectable* endogenous interferon is required to trigger the protective mechanism (5). Strong indirect evidence that interferon synthesis might be required at low concentrations of poly I:C was reported by Schafer and Lockart (4). In the present study, however, data are presented which suggest that this is not always the case. McCoy cells which were protected by interferon were apparently unable to produce it. Yet, these cells were protected by poly I:C. It appears, therefore, that in McCoy cells synthesis of demonstrable interferon is not an essential intermediate step for the development of the antiviral state. The suppression of antiviral activity of interferon by metabolic inhibitors (11) has been interpreted to mean that interferon acts as a derepressor for the synthesis of an antiviral protein. Poly I:C could induce the resistant state either by directly stimulating the synthesis of the hypothetical antiviral protein or by another as yet undefined mechanism.

The possibility exists, of course, that McCoy cells produce interferon at concentrations below the sensitivity of our assay system. Until more sensitive methods for the assay of interferon are developed, however, we must assume that no interferon was produced. Furthermore, it is hard to visualize how such minute amounts of interferon induce complete resistance, while relatively large amounts of exogenous interferon result in only partial protection.

That poly I:C can act through mechanisms other than interferon induction was suggested by Kjeldsberg and Flikke (12). In their study, poly I:C interfered directly with virus synthesis by binding to the virus-specific RNA polymerase. We suggest that induction of interferon is but one of several mechanisms of action of poly I:C and that the mechanism utilized is dependent upon the cell system used.

Summary. Data are presented which show that McCoy cells do not produce detectable levels of interferon but respond to the protective action of either interferon or poly I:C. Protection by poly I:C is blocked by actinomycin D treatment, suggesting that an active cellular process is required. It is concluded that, in McCoy cells, poly I:C acts through a

mechanism other than interferon induction.

1. Field, A. K., Tytell, A. A., Lampson, G. P., and Hilleman, M. R., Proc. Nat. Acad. Sci. U.S.A. **61**, 340 (1968).

2. Vilcek, J., NG, M. H., Friedman-Kein, A. E., and Krawciw, T., J. Virol. **2**, 648 (1968).

3. DeClercq, E., and Merrigan, T. C., Nature (London) **222**, 1148 (1969).

4. Schafer, T. W., and Lockart, R. Z., Jr., Nature (London) **226**, 450 (1970).

5. Vilek, J., and Varacalli, F., J. Gen. Virol. **13**, 185 (1971).

6. Grimes, J. F., Moore, R. W., and Grumbles, L. C., Avian Dis. **132**, 345 (1969).

7. Eagle, H., Science **122**, 501 (1955).

8. Giron, D. J., and Pindak, F. F., Appl. Microbiol. **1**, 811 (1969).

9. Giron, D. J., Appl. Microbiol. **18**, 584 (1969).

10. Allen, P. T., and Giron, D. J., Appl. Microbiol. **20**, 317 (1970).

11. Taylor, J., Biochem. Biophys. Res. Commun. **14**, 447 (1964).

12. Kjeldsberg, E., and Flikke, M., J. Gen. Virol. **10**, 147 (1971).

Received May 17, 1972. P.S.E.B.M., 1972, Vol. 141.