Mink Parvoviruses and Interferons: In Vitro Studies

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Although interferons can inhibit the replication of a number of viruses, little is known about their ability to inhibit parvovirus replication. Therefore, in vitro experiments were done to determine if Aleutian disease virus and mink enteritis virus, two autonomously replicating mink parvoviruses, (i) induced interferon, (ii) were sensitive to the effects of interferon, or (iii) inhibited the production of interferon. The results indicated that these parvoviruses neither induced nor were sensitive to the effects of interferon. Furthermore, preexisting parvovirus infections did not inhibit poly(I) · poly(C)-induced interferon production. This independence from the interferon system may, therefore, be a general property of the autonomously replicating parvoviruses.

Interferons are a family of proteins and glycoproteins that play an important, early role in limiting virus yield and spread (2, 3). Indeed, interferon production constitutes the earliest host response to virus infection and is usually detectable within hours of exposure, well before inflammatory, humoral, or cell-mediated immune responses occur (6, 12). However, the role of interferon in parvovirus infections is not understood.

Studies of Aleutian disease virus (ADV)-infected mink suggest that the administration of poly(I) · poly(C), a potent interferon inducer, has no effect on the progression of Aleutian disease (18) and that ADV-infected mink do not produce interferon even when virus replication is maximal (23). However, the presence of antibodies to poly(I) · poly(C) and high nuclease activities in the sera from normal mink (18) have led to difficulties in evaluating and understanding the in vivo situation. Therefore, we examined the replication of two mink parvoviruses in vitro in two different cell lines to determine whether (i) these viruses can induce interferon, (ii) the addition of exogenous interferon has any effect on virus replication, and (iii) a preexisting parvovirus infection results in the inhibition of poly(I) · poly(C)-induced interferon production.

To determine whether the two cell lines, mink lung cells ATCC CCL-64 and feline kidney (CRFK) cells (9, 16) were capable of producing interferon, we challenged these cells with various concentrations of the synthetic polyribonucleotide poly(I) · poly(C)-DEAE dextran as previously described (D. L. Wiedbrauk and G. R. Burleson, J. Interferon Res., in press). The resulting cell-free supernatant fluids were assayed for antiviral activity on CCL-64 or CRFK cell monolayers by using a standard cytopathic effect bioassay (1) and a vesicular stomatitis virus (VSV) challenge of 15 PFU per well. The optimum concentration for the induction of interferon in CCL-64 cells was 100 µg of poly(I) · poly(C)-200 µg of DEAE dextran per ml, whereas CRFK cells required 200 µg of poly(I) · poly(C)-400 µg of DEAE dextran per ml (data not shown). These inducer concentrations were used in all subsequent experiments.

The kinetics of interferon production was established with these inducers to determine when maximal interferon yields were achieved. In CCL-64 cell cultures incubated at 37°C,

antiviral activity was first detected 8 h after the addition of the inducer, and maximum titers were achieved after 24 h (Fig. 1). In contrast, lower interferon yields were obtained from similarly treated CRFK cells. These results were not unique to the interferon inducer, because similar results were obtained with Newcastle disease virus-infected CRFK cells (24; unpublished observations). Because ADV requires a temperature of 31.8°C for replication (16), the interferon response also was determined at this temperature. In CCL-64 and CRFK cells incubated at 31.8°C, interferon production was delayed; antiviral activity was first detected 24 h after induction, and maximum levels were reached after 48 h. In contrast to the results from experiments done at 37°C, some antiviral activity remained in the supernatant fluids 72 h after induction, but none was detected at 96 h (Fig. 1).

Physical characterization of the poly(I) · poly(C)-DEAE dextran-induced interferons indicated that both CCL-64 and CRFK cells produced typical type I interferons that had little if any antiviral activity on heterologous cells (Table 1). The antigenic characteristics of the CCL-64 and CRFK cell interferons, however, appeared to be quite different from those of human leukocyte interferon and mouse L-cell (α/β) interferon because antisera to these interferons failed to neutralize the antiviral activity produced by CCL-64 and CRFK cells.

Because many viruses are good interferon inducers (22), we determined whether two mink parvoviruses, the Gorham strain of ADV (ADV-G) (5) and mink enteritis virus (MEV) (12, 19), could induce interferon production in CCL-64 and CRFK cells. The results indicated that neither virus induced detectable levels of interferon in these cells (Fig. 1). These results occurred irrespective of the multiplicity of infection or the temperature of incubation. We can speculate that this inability to induce interferon production could be advantageous in vivo, because it would allow these viruses to evade the antiviral and immunomodulatory actions of interferon and to more readily establish an infection.

Experiments were done to determine whether ADV-G and MEV were sensitive to the effects of interferon. CCL-64 and CRFK cells were treated with interferons from homologous cells or with human leukocyte interferon (Lee Biomolecular, San Diego, Calif.) and then challenged with either ADV-G, MEV, or VSV. After infection, virus yields from interferon-pretreated and control monolayers were compared. Data

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FIG. 1. Kinetics of interferon production in CCL-64 cells (top) and CRFK cells (bottom) after induction with poly(I) · poly(C)-DEAE dextran, ADV-G, or MEV. Cell monolayers were incubated with the poly(I) · poly(C)-DEAE dextran inducer, ADV-G, or MEV (virus multiplicities of infection were 10, 1.0, or 0.1) for 2 h at the indicated temperatures. The interferon inducer was removed with two phosphate-buffered saline washes, Eagle minimum essential medium supplemented with fetal bovine serum was added, and the cells were incubated as before for the indicated times. The cell-free supernatant fluids were assayed for antiviral activity on homologous cells. Because ADV-G and MEV did not induce interferon in these cells under any of the conditions imposed, the data for each cell type are shown as a single line. The variability of the interferon assay was 1 twofold dilution.

from a representative experiment showed that pretreating the cells with interferon had no effect on ADV-G and MEV yields (Table 2). VSV replication, however, was extremely sensitive to interferon pretreatment; as few as 10 U of interferon inhibited VSV replication 100,000-fold. Although at extremely high interferon concentrations ADV-G and MEV yields were reduced, this reduction was not considered significant, because high concentrations of interferons (100 to 1,000 U/ml) have been shown to inhibit cell growth (21) and delay the entry of cells into the S phase (8); also, factors present in the S phase are required for parvovirus replication (for a review, see reference 4).

Finally, we determined whether a preexisting parvovirus infection could impair interferon production. CRFK and CCL-64 cells were infected with ADV-G or MEV for various

lengths of time and then incubated with the poly(I) · poly(C)-DEAE dextran inducer. The cell-free supernatant fluids were collected 24 h after interferon induction and assayed for antiviral activity. The results indicated that a preexisting infection did not interfere with poly(I) · poly(C)-DEAE dextran-induced interferon production in CCL-64 (Table 3) or CRFK (Table 4) cells. These data supported similar results from parvovirus-infected mink that suggested that supervening ADV infections do not influence poly(I) · poly(C)-induced interferon induction even when virus replication is maximal (23). Therefore, these observations indicated that ADV-G and MEV infections did not inhibit interferon-mediated host cell functions in vitro and further emphasized the independence of these parvoviruses from the production and action of interferon.

ADV and MEV are two unrelated members of the family *Parvoviridae* (20) that produce different types of disease (10, 13, 14, 17, 19). ADV-G and MEV also exhibit different properties when cultivated in vitro. ADV does not replicate well in cell culture, and although a few virus isolates have been propagated in this manner, cell culture-adapted strains may have altered virulence for mink (5). Furthermore, ADV causes cytopathic effects only at high multiplicities of infection (unpublished observations), and there is no evidence that ADV can spread from cell to cell (5, 16). In contrast, MEV replicates well in cell culture, produces cytopathic effects, spreads efficiently from cell to cell, and retains its virulence for mink (15).

Although ADV-G and MEV possess different biologic characteristics, they behaved similarly with respect to the

TABLE 1. Physical characteristics of CRFK and CCL-64 cell interferons induced by poly(I) · poly(C)-DEAE dextran^a

Treatment or condition	Interferon reciprocal dilution ^b	
	CRFK	CCL-64
None	64	2,048
pH 2.0 ^c	64	2,048
56°C for 1 h	64	2,048
Trypsin ^d	<4	<4
RNase ^e	64	2,048
Dialysis ^f	64	2,048
Centrifugation ^g	64	2,048
Antiviral activity		
CRFK cells	64	<4
CCL-64 cells	16	2,048

^a CCL-64 interferon was induced by the addition of 100 µg of poly(I) · poly(C) per ml–200 µg of DEAE dextran per ml to preformed cell monolayers. The cells were exposed to the inducer for 1 h at 37°C and were then removed with three phosphate-buffered saline washes. Media were added to the monolayers, and the cells were incubated for 24 h at 37°C. Cell-free supernatant fluids were collected and used as described below. The CRFK cell interferon was obtained as described above, except that the inducer contained 200 µg of poly(I) · poly(C) per ml–400 µg of DEAE dextran per ml. Neither CRFK nor CCL-64 interferon was neutralized by antiserum to human leukocyte or mouse L-cell interferon.

^b Numbers represent the reciprocal of the dilution that protected 50% of the cell monolayer from VSV-induced cytopathic effects. The variability of this assay was 1 twofold dilution.

^c Samples were dialyzed against 0.15 M HCl–KCl (pH 2.0) for 24 h at 4°C. The pH was neutralized by dialysis against Hanks balanced salt solution (pH 7.4) for 16 h at 4°C.

^d GIBCO trypsin (1:250) was added to the interferon to a final concentration of 1 mg/ml. After the preparation was incubated for 1 h at 37°C, 1.0 mg of soybean trypsin inhibitor was added.

^e Bovine pancreatic RNase A in 1 mM EDTA was added to 1 ml of interferon to give a final concentration of 50 µg/ml. After the preparation was incubated for 1 h at 37°C, the pH was adjusted to 7.4 with 0.1 N NaOH.

^f *M_r* cutoff of 14,000.

^g At 100,000 × *g* for 4 h.

TABLE 2. Virus yield in cell monolayers pretreated with interferon^a

Cell line and amt of interferon (U) added	Virus yield		
	ADV-G (FFU ^b ± SE)	MEV (FFU ^b ± SE)	VSV (PFU ^c ± SE)
CRFK			
1,000	$8.0 \times 10^6 \pm 12,500$	$5 \times 10^6 \pm 7,400$	$1.1 \times 10^1 \pm 9$
100	$1.2 \times 10^7 \pm 8,870$	$4 \times 10^6 \pm 10,400$	$1.9 \times 10^1 \pm 11$
10	$5.1 \times 10^7 \pm 9,680$	$8 \times 10^7 \pm 11,400$	$4.3 \times 10^2 \pm 9,890$
0	$6.3 \times 10^7 \pm 11,400$	$6 \times 10^7 \pm 9,700$	$7.8 \times 10^7 \pm 3,050$
CCL-64			
1,000	$1.1 \times 10^4 \pm 570$	$9 \times 10^6 \pm 4,760$	$1.5 \times 10^1 \pm 10$
100	$1.8 \times 10^4 \pm 660$	$8 \times 10^6 \pm 6,340$	$2.1 \times 10^2 \pm 14$
10	$4.3 \times 10^4 \pm 790$	$4 \times 10^7 \pm 5,175$	$4.0 \times 10^3 \pm 157$
0	$3.5 \times 10^4 \pm 830$	$4 \times 10^7 \pm 4,970$	$8.2 \times 10^8 \pm 3,550$

^a Triplicate cell monolayers were treated with interferon for 24 h at 37°C, washed twice with phosphate-buffered saline, and infected with 6×10^4 focus-forming units of ADV-G or MEV or 8×10^4 PFU of VSV. The cells were lysed and the virus was extracted as previously described (5).

^b FFU, Focus-forming units on CRFK cells. The amounts of ADV-G shown for the CCL-64 cells represent carryover virus, since these levels represent no increase over virus yields measured 4 h after infection.

^c PFU on BHK-21 cells.

interferon system. Neither virus induced detectable levels of interferon in CCL-64 or CRFK cells, nor was their replication affected when these cells were pretreated with exogenous interferon. Engers et. al. (11), in the only other report dealing with interferons and parvoviruses in vitro, suggested that pretreating cell cultures with exogenous

murine α - or γ -interferon does not inhibit the replication of the minute virus of mice. Thus, a general characteristic of all autonomously replicating parvoviruses may be their ability to replicate regardless of the effects of the interferon system.

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TABLE 3. Effect of parvovirus infections on interferon production in CCL-64 cells^a

Cells	Interferon reciprocal dilution with or without inducer (by h of virus infection) ^b			
	+ Poly(I) · poly(C)		- Poly(I) · poly(C)	
	24	48	24	48
ADV-G infected	256	64	<4	<4
MEV infected	256	128	<4	<4
Uninfected	256	128	<4	<4

^a CCL-64 cell monolayers were infected with 6×10^5 focus-forming units of either ADV-G or MEV for 24 or 48 h and then treated with 100 μ g of poly(I) · poly(C) per ml-200 μ g of DEAE dextran per ml. Cell-free supernatant fluids were harvested 24 h after interferon induction and assayed for antiviral activity on new CCL-64 cell monolayers. The variability of the interferon assay was 1 twofold dilution.

^b Numbers represent the reciprocal of the dilution of the supernatant fluids that protected 50% of new CCL-64 cell monolayers from virus-induced cytopathic effects.

TABLE 4. Effect of parvovirus infections on interferon production in CRFK cells^a

Cells	Interferon reciprocal dilution with or without inducer (by h of virus infection) ^b			
	+ Poly(I) · poly(C)		- Poly(I) · poly(C)	
	24	48	24	48
ADV-G infected	64	64	<4	<4
MEV infected	32	32	<4	<4
Uninfected	64	32	<4	<4

^a CRFK cell monolayers were infected with 6×10^5 focus-forming units of either ADV-G or MEV for 24 or 48 h and then treated with 200 μ g of poly(I) · poly(C)-400 μ g of DEAE dextran per ml. Cell-free supernatant fluids were harvested 24 h after interferon induction and assayed for antiviral activity on new CRFK cell monolayers. The variability of the interferon assay was 1 twofold dilution.

^b Numbers represent the reciprocal of the dilution of the supernatant fluids that protected 50% of new CRFK cell monolayers from virus-induced cytopathic effects.

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