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Role of Interferon in Murine Lactic Dehydrogenase Virus Infection, In Vivo and In Vitro

HERMAN DUBUY, SAMUEL BARON, CAROL UHLENDORF, AND MARTIN L. JOHNSON
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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The induction of interferon (IF) by lactic dehydrogenase virus (LDV) and the action of exogenous IF on LDV multiplication have been studied in vivo and in vitro. In vivo the induction of serum IF by LDV was shown to correspond with the virus challenge dose. In vitro LDV-infected macrophages did not produce detectable IF. As to the action of IF in vivo, the prophylactic or therapeutic administration of IF to mice reduced the LDV serum titers only temporarily. In vitro, (i) the sensitivity of LDV to IF in macrophage culture was 30 times less than that of vesicular stomatitis virus, and 5 times less than that of Sendai virus, and (ii) prolonged exposure of LDV-infected macrophages to high IF concentrations did not cure the chronic infection, but only decreased the virus titer. The probable role of IF in the establishment of the early phase of a persistent but reduced viremia in mice is discussed.

The chronic viremia of mice caused by the lactic dehydrogenase virus (LDV) probably is due to the lifelong LDV infection of the mouse macrophages (5, 19). This LDV macrophage system presents a useful model to answer questions regarding the conditions under which certain chronic infections can be eliminated by host defenses such as interferon (IF). This requires information on the induction of interferon production by LDV, and on the relative sensitivity of LDV to the action of mouse interferon, both in vivo and in vitro.

It has been previously shown that, in vivo, LDV infection induced the production of moderate amounts of IF (3, 4, 9) and that large amounts of Newcastle disease virus (NDV)-induced IF retarded LDV viremia (4). In vitro, no measurable induction of IF by LDV could be found (4, 8) although exogenous IF decreased the LDV titer in this macrophage system (8). However, these effects of IF in vivo and in vitro have not been determined quantitatively, nor has the sensitivity of LDV to IF been compared to that of other viruses. This paper will present data in four sections: (i) induction of IF by LDV in vivo, (ii) action of IF on LDV in vivo, (iii) induction of IF by LDV in vitro in macrophages, and (iv) action of IF on LDV in vitro. The place of the persistent LDV infection among three groups of peristent infections, based on the role of IF, will be discussed.

MATERIALS AND METHODS

Animals. Random-bred Swiss albino mice were supplied by the National Institutes of Health animal production center.

Viruses. The strain of LDV used was isolated from a naturally infected Ehrlich ascites carcinoma, carried in CDF_1 mice. The virus was titered by measuring the rise in blood plasma lactic dehydrogenase (LDH) level as described before (4, 6). The titers (log_{10} mean infective dose [ID_{50}]/ml) and the corresponding standard deviations were determined by applying the Spearman-Karber method (4, 10) to the numbers of mice estimated to be infected by intraperitoneal (i.p.) injection of serial dilutions of the unknown serum.

Vesicular stomatitis virus (VSV), Indiana strain, was prepared in chicken embryo monolayer cells (1), and the plaque titration of the stock suspension was done in mouse cells (approximately $10^{7.5}$ plaque-forming units [PFU]/ml).

Sendai virus. A mouse lung-adapted strain was prepared in the allantoic cavity of 11-day-old embryonated hen eggs, and the stock suspension contained approximately $10^{9.0}$ PFU/ml, as assayed on monolayers of African green monkey kidney cells (approximately 1,000 hemagglutination [HA] units/ml). Hemadsorption was read as a 1 to 4 scale (11). Coded cultures were periodically read to verify the reliability of the classification in four hemadsorption groups.

Interferon. The interferon was induced by NDV in C243 cells, a cloned mouse cell line (18). The interferon was harvested after 24 h, treated at pH 2 for 5 days, and then readjusted to pH 7.0. When tested by the GD-7 virus hemagglutinin yield reduction method

on mouse L cells (17), the stock IF titered about 4×10^4 units per ml. The mouse reference IF titered 3×10^4 units/ml. IF control fluid was prepared in the same manner from the supernatants of uninfected cultures.

Media. Collecting medium consisted of the following: medium 199 with SPAM (streptomycin, 100 μg/ml; penicillin, 100 units/ml; acromycin, 10 μg/ml; mycostatin: 25 units/ml) and heparin, 1 USP unit/ml. Wash medium consisted of medium 199 with SPAM for LDV, or Earle balanced salt solution for VSV or Sendai virus. Growth medium consisted of medium 199, SPAM, and 20% fetal bovine serum (FBS).

Macrophages. Unstimulated mouse macrophages were obtained from female Swiss albino mice by injecting, after decapitation, 3 to 4 ml of collecting medium i.p., massaging gently, and removing the fluid through a ventral incision with a Pasteur pipette. The suspension was kept on ice. After proper dilution, 1-ml samples containing approximately 2.5×10^6 cells per ml were distributed into open test tubes, held vertically in a spring rack, and covered loosely with a single metal lid (2). After 1 h of incubation in a CO_2 chamber at 37 C, the nonadhering cells were removed by washing the tubes with wash medium. The adherent cells (macrophages) were reincubated after addition of 1 ml of growth medium per tube until used.

RESULTS

Induction of IF by LDV in vivo. Preliminary data on the induction of interferon by LDV in mice have been presented previously (4). Figure 1 presents the kinetics of induction after i.p. inoculation with LDV of either a low dose (10^3 ID_{50}/mouse) or a high dose (10^7 ID_{50}/mouse). It can be seen that measurable serum interferon is produced 12 h after injection with a high dose, with a maximum at 24 h. With a low dose, IF appears in the serum 24 h after injection, reaching a peak at 30 h; the virus titers start to decline only after the IF peak has been reached.

Action of IF on LDV replication in vivo. Preliminary data on the action of IF on LDV replication in mice have been presented previously (4). It was reported that a single induction of IF by NDV, in mice which had been inoculated with LDV 14 days previously, temporarily decreased the LDV titer. In the present experiments exogenous IF was administered prophylactically and therapeutically. The prophylactic effect was studied after pretreatment with a total of at least 2×10^4 units of IF per mouse, either by the i.p. or the intravenous (i.v.) route. Table 1 presents the results of three experiments, with different multiplicity of infection (MOI) and varying IF treatments. The table indicates that, as was found with NDV-induced IF, the LDV titers were only reduced during the early stages of infection.

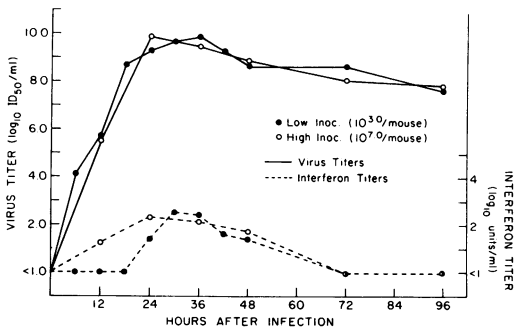

FIG. 1. *Induction of serum interferon in mice, after i.p. inoculation with LDV, either at a low dose ($10^3 ID_{50}$/mouse), or a high dose ($10^7 ID_{50}$/mouse).*

The therapeutic effect of exogenous IF in vivo was studied by preinfection of mice with LDV, followed by i.p. or i.v. administration of IF (Table 2). Mice were preinfected with a dose of 10^6 ID_{50} of LDV per mouse, 7, 5, 4, 3, 2 and 1 day(s) before i.v. administration of either one dose of 0.3 ml of IF (10^4 units per ml) or of 0.3 ml of control fluid of cells used to produce the IF. The high dose of LDV inoculum was used to insure maximum virus titers within 24 h, the time of the first treatment. (The same peak would have been reached 6 to 12 h later with a small dose.) Blood samples were taken at different times after virus inoculation for the determination of LDV titers. Table 2 shows that no significant differences were found between the LDV titers of the treated and the corresponding control groups. In another experiment employing repeated injections of IF (Table 3), three groups of mice were injected i.p. with 10^7 ID_{50} of LDV per mouse. At 5 days after inoculation with LDV, one group received two doses of 16×10^4 units of IF i.p. per day for 4 consecutive days, and one group received two doses of IF i.p. (16×10^4 units) and i.v. (3.2×10^4 units) per day. One group received two doses per day of control fluid. Table 3 shows that, with higher doses of IF, a significant, but temporary decrease in virus titer occurred only in the group which received i.p. and i.v. injections of IF.

Induction of IF in macrophages, in vitro, by LDV. We have reported previously and now confirm that supernatants from unstimulated mouse macrophage cultures infected with LDV did not contain measurable IF (5, p. 993). Further, LDV-infected macrophages did not produce measurable interferon or manifest resistance to VSV or Sendai virus superinfection since there was no significant reduction in VSV-CPE or Sendai virus hemadsorption in macrophages preinfected with LDV, as compared to LDV-uninfected macrophages (see Ta-

TABLE 1. *Prophylactic action of IF on LDV replication in vivo*

Expt	Treatment	Virus titers (log_{10} ID_{50}/ml ± SD) of blood plasma				
		12[a]	24	36	48	84
1[b]	Control	5.5 ± 0.20	9.1 ± 0.28	9.5 ± 0.28	8.5 ± 0.20	
	IF	4.5 ± 0.28	9.1 ± 0.24	8.9 ± 0.25	9.1 ± 0.25	
2[c] A	Control	<3.0	9.7 ± 0.20	8.3 ± 0.20		
	IF	2.5 ± 0.37	8.5 ± 0.28	9.3 ± 0.20		
B	Control	3.3 ± 0.20	9.9 ± 0.32	9.1 ± 0.25		
	IF	2.7 ± 0.32	9.3 ± 0.32	9.7 ± 0.20		
3[d]	Control	4.5 ± 0.28	8.9 ± 0.25	9.1 ± 0.37	8.9 ± 0.25	7.7 ± 0.20
	IF, i.p.	3.7 ± 0.32	9.5 ± 0.20	9.5 ± 0.20	9.3 ± 0.20	7.9 ± 0.32
	IF, i.v.	3.5 ± 0.28	8.9 ± 0.29	9.5 ± 0.20	8.5 ± 0.28	7.3 ± 0.20

[a] Hours after inoculation.

[b] One milliliter of IF, 10^4 units/ml, injected i.p. at 18 h and again 1 h before i.p. injection of 100 ID_{50} of LDV/mouse.

[c] One milliliter of IF, 10^4 units/ml, injected at 9 h and at 1 h before i.p. injection of 10 ID_{50} of LDV/mouse in expt 2A, and 100 ID_{50} of LDV/mouse in expt 2B.

[d] The i.p. injected group received 1 ml and the i.v. group received 0.3 ml of IF, 3×10^4 units/ml, at 13, 9, 3, and 1 h before, and 3, 12, and 19 h after i.p. injection of 10 ID_{50} of LDV/mouse.

TABLE 2. *Therapeutic action of a single dose of IF in vivo administered at different time intervals after LDV inoculation*

Inoculation	Virus titers (log_{10} ID_{50}/ml ± SD) of pooled blood plasma (log_{10} ID_{50}/ml) of 5 mice[a]						
	7[b]	5	4	3	2	1	0
LDV	7.5 ± 0.20	7.7 ± 0.32	7.5 ± 0.35	7.9 ± 0.25	7.7 ± 0.32	9.1 ± 0.40	8.7 ± 0.20
LDV + IF	7.1 ± 0.28	7.9 ± 0.25	7.3 ± 0.35	7.5 ± 0.20	7.7 ± 0.35	9.3 ± 0.32	8.9 ± 0.32

[a] At 12 h after administration of IF or control fluid.

[b] Intervals, in days, between LDV inoculation (10^6 ID_{50}/mouse, i.p.) and IF administration.

TABLE 3. *Therapeutic action of repeated IF administrations, in vivo, starting 5 days after LDV inoculation*[a]

Administration	Virus titers ($log_{10}ID_{50}$/ml ± SD) of blood plasma[b] at various times after the first administration of IF[c]					
	1[d]	2	3	4	6	8
Control fluid	8.5 ± 0.20	7.1 ± 0.28	7.5 ± 0.28	7.7 ± 0.32	6.5 ± 0.28	6.9 ± 0.25
IF, i.v.	7.9 ± 0.25	7.3 ± 0.20	7.1 ± 0.32	7.3 ± 0.20	7.1 ± 0.25	6.5 ± 0.20
IF, i.v. + i.p.	7.1 ± 0.32	6.3 ± 0.20	6.3 ± 0.32	5.9 ± 0.25	6.7 ± 0.20	>7.5

[a] Mice inoculated i.p. with 10^7 ID_{50} of LDV/mouse.

[b] Blood samples collected 1 h before IF administration.

[c] Each i.v. injection—0.2 ml, approximately 3.2×10^4 units of IF; each i.p. injection—1.0 ml, approximately 16×10^4 units of IF, twice per day for 4 days.

[d] Days after first administration of IF.

bles 7, 8, and 9). This raised the question whether the macrophages under our culture conditions could produce IF. Therefore, attempts were made to induce measurable IF by infecting the cultured macrophages with NDV or Chikungunya virus, or by adding poly I poly C ± diethylaminoethyl-dextran. Only NDV infection produced some measurable IF (approximately 10 units/ml of supernatant), and poly I poly C, although not producing measurable IF, induced some protection of the macrophages against VSV and Sendai virus (a more sensitive test of IF induction [11]). Thus the cultured macrophages are capable of producing detectable IF in response to inducing agents other than LDV. Since LDV did not induce measurable IF in macrophage cultures, experiments were performed to determine whether

homologous interference occurred. Macrophage cultures were obtained from chronic LDV-infected mice. After in vitro superinfection with LDV (Table 4) the superinfected macrophages showed a reduced LDV titer when compared with macrophages from control mice, but an increased titer when compared with the titer of unchallenged macrophages from chronically infected mice. These results are compatible with the occurrence of homologous interference in superinfected macrophages (see Discussion).

Action of IF on LDV replication in vitro. To establish the relative sensitivity of LDV, as compared to VSV and Sendai virus to IF, the prophylactic action of increasing amounts of mouse interferon on these viruses, which all replicate in mouse macrophages, was determined. The macrophage cultures, 1 day after harvest, were preexposed for 16 to 18 h to a series of 10-fold dilutions of a stock solution of interferon, washed once, and then infected with either approximately 10^8 ID_{50} of LDV per tube (MOI > 100), $10^{6.5}$ PFU of VSV per tube (MOI > 3), or $10^{8.5}$ ID_{50} Sendai virus per tube (MOI > 100). To remove unabsorbed virus, the LDV series were washed five times, 1 h after inoculation, and thereafter the combined supernatant samples of at least three tubes were collected for virus titration, at 12, 24 and 36 h after infection. The VSV and Sendai virus cultures were washed three times, 1 h after inoculation, refed with growth medium, and reincubated for 48 h before examination for cytopathic effect (CPE) or determination of degree of hemadsorption. The sensitivity of LDV to interferon was measured by the highest IF dilution which caused a minimally significant decrease (one $\log_{10}$) in the LDV titer, as compared with the LDV titer of the corresponding control. The sensitivity of VSV was measured by the highest IF dilution which gave 50% protection of the macrophages against VSV CPE, and the sensitivity of Sendai virus was measured by the highest IF dilution which gave 50% protection against hemadsorption of human O erythrocytes by the macrophages. The data are presented in Table 5 and are analyzed further in Table 6. It can be seen that the relative sensitivity of the three viruses to interferon, in decreasing order, was as follows: VSV, Sendai virus, and LDV. Thus, LDV is about 30 times less sensitive to interferon than is VSV, and 5 times less sensitive than Sendai virus, when tested in the mouse macrophage system in vitro.

Next, the question was asked whether preinfection with LDV would render the macrophages unresponsive to the protective action of IF against superinfecting viruses. Macrophages, either preinfected in vitro with LDV for 6 days or uninfected, were exposed to one dose of exogenous IF in the amounts found to inhibit the respective viruses. The macrophages were then superinfected or sham-infected with LDV 12 h after, or with VSV or Sendai virus 6 h after addition of the IF. The results (Tables 7, 8, and 9) show that preinfection with LDV did not render the macrophages unresponsive to the action of exogenous IF; the IF reduced the titers of the three superinfecting viruses. It should be noted that the LDV titers are relatively low 6 days after the primary infection.

The data on the in vitro therapeutic effect of multiple doses of exogenous IF on LDV infection of macrophage cultures are presented in Table 10. It can be seen that the prolonged exposure of LDV-infected macrophage cultures

TABLE 4. *Replication of LDV in macrophages from uninfected and from chronically LDV-infected mice, superinfected with 10^8 ID_{50} of LDV/ml, 24 h after start of cultures*

Expt	Origin of macrophages	Virus titers ($\log_{10}$ ID_{50}/ml ± SD) of supernatants of macrophage cultures at various times after infection					
		Orig. sup.[a]	5th wash[b]	12 h	24 h	48 h	72 h
1	Control		2.9 ± 0.25	5.9 ± 0.25	5.7 ± 0.20	5.1 ± 0.25	
	Chronic[c] + LDV superinf.		3.9 ± 0.25	4.7 ± 0.20	4.5 ± 0.20	3.1 ± 0.32	
	Chronic	3.9 ± 0.25					
2	Control		3.5 ± 0.20	5.9 ± 0.25	6.9 ± 0.25	6.7 ± 0.20	6.5 ± 0.28
	Chronic[d] + LDV superinf.		4.3 ± 0.20	5.5 ± 0.20	5.5 ± 0.28	6.1 ± 0.25	5.9 ± 0.32
	Chronic	3.5 ± 0.37		2.9 ± 0.32	3.3 ± 0.32	3.9 ± 0.28	3.5 ± 0.28

[a] Orig. sup., Supernatant of macrophages before superinfection.
[b] 5th wash, Supernatant of macrophages after 4 washes, 1 h after LDV infection.
[c] Chronically infected for 7 months.
[d] Chronically infected for 74 days.

TABLE 5. *Relative sensitivity of LDV, VSV, and Sendai virus to exogenous infection in mouse macrophage culture*[a]

Expt	Concn of IF[b] (units/tube)	LDV yield (log_{10} ID_{50}/ml ± SD) at			VSV CPE (%) at 48 h	Sendai virus hemadsorption (%) at 48 h
		12 h	24 h	36 h		
1	Control	7.5 ± 0.20	8.3 ± 0.32	7.9 ± 0.25	100	ND[c]
	1	7.3 ± 0.32	8.5 ± 0.28	7.7 ± 0.20	50	
	10	6.7 ± 0.35	8.3 ± 0.32	7.5 ± 0.28	0	
	100	6.7 ± 0.20	7.1 ± 0.25	6.9 ± 0.32	0	
2	Control	6.3 ± 0.20	7.1 ± 0.25	6.9 ± 0.25	100	100
	1	6.3 ± 0.20	6.7 ± 0.40	7.3 ± 0.20	75	75
	10	5.5 ± 0.28	6.3 ± 0.32	6.9 ± 0.25	25	50
	100	5.5 ± 0.28	6.3 ± 0.20	6.5 ± 0.35	0	25
	1000	4.7 ± 0.20	5.7 ± 0.20	6.3 ± 0.32	0	0
3	Control	6.3 ± 0.35	7.7 ± 0.20	7.5 ± 0.28	100	100
	1	6.1 ± 0.28	7.5 ± 0.20	7.9 ± 0.25	100	100
	10	6.5 ± 0.28	7.7 ± 0.20	7.1 ± 0.32	40	75
	100	5.5 ± 0.35	6.1 ± 0.28	6.1 ± 0.42	0	10
	1000	4.5 ± 0.20	5.1 ± 0.37	5.9 ± 0.40	0	0

[a] Viruses added 24 h after start of cultures. LDV, 10^8 ID_{50}/ml; VSV, 10^5 ID_{50}/ml (titered in mouse L cells); Sendai virus, 10^8 ID_{50}/ml (titered in AGMK cells).

[b] IF administered 16 to 18 h before virus infection.

[c] ND, Not done.

TABLE 6. *Minimum number of IF units per tube causing inhibition of LDV, VSV, and Sendai virus replication in macrophage cultures*

Expt	LDV	VSV	Sendai virus
1	100	1	ND[a]
2	160	3	10
3	50	6	30
Avg	103	3.3	20

[a] ND, Not done.

decreased the virus titer, without eliminating the virus during the 6 days of exposure. After this period a progressive toxic CPE was observed in the sets treated with IF.

DISCUSSION

It is clear from the present findings that LDV belongs to a class of poor interferon inducers. We found that in vivo a plateau of maximum circulating interferon production of about 100 units of interferon/ml of plasma occurred from 24 to 36 h after infection, and no measurable interferon occurred 72 h after infection. Increasing the size of virus inoculum led to an earlier appearance of interferon beginning at about 12 h (Fig. 1), which paralleled the earlier decline of viremia.

The in vivo action of interferon on LDV multiplication was determined by a study of the prophylactic and the therapeutic action of exogenously supplied interferon. The experiments on the prophylactic action of interferon (Table 1) show that LDV titers were only temporarily reduced to a small but significant degree, between 12 and 24 h, followed by a rise to control or greater-than-control levels. Apparently the interferon concentration at the locus of LDV multiplication, the macrophages, was not high enough to strongly inhibit LDV, which is relatively interferon insensitive. Further, postinfection treatment of LDV-infected mice with IF showed no significant decline of virus titers (Table 2).

In vitro LDV infection of macrophages does not lead to IF induction. The absence of measurable IF induction in macrophages infected with LDV in vitro and the susceptibility of macrophage to superinfection confirm that LDV is a poor IF inducer, and that the small in vivo production of IF may occur in a cell type other than the macrophages, which are the primary target cells for LDV. The data on the in vitro action of IF on LDV replication confirm the insensitivity of LDV, found in vivo. The data demonstrate this low sensitivity in two ways: (i) by showing that the sensitivity of LDV to IF was 30 times less than that of VSV, and 5 times less than that of Sendai virus, and (ii) by showing that prolonged exposure of LDV-infected macrophages to IF did not effect a cure, but only a decrease in virus titer.

Thus, LDV is a poor and temporary inducer of IF, and it has a low sensitivity to the antiviral

TABLE 7. *Responsiveness of LDV-carrying macrophages to the action of interferon on superinfecting LDV*[a]

Macrophages infected with:	Superinfected with:	LDV titers (log_{10} ID_{50}/ml ± SD) of supernatants		
		12[b]	24	36
6 days LDV	Sham	4.9 ± 0.37	5.3 ± 0.32	5.3 ± 0.40
6 days LDV + IF (1,000 units)[c]	Sham	4.1 ± 0.32	4.3 ± 0.20	4.5 ± 0.37
6 days LDV	LDV	4.9 ± 0.32	5.3 ± 0.35	5.3 ± 0.35
6 days LDV + IF (1,000 units)	LDV	3.5 ± 0.28	4.3 ± 0.35	4.1 ± 0.40

[a] Preinfection with LDV with an MOI of approximately 10. Superinfected after 6 days with LDV, 10^8 ID_{50}/ml.
[b] Hours after sham or superinfection.
[c] Numbers in parentheses give the units of interferon per tube added to the macrophage cultures, 12 h before sham or superinfection with LDV.

TABLE 8. *Responsiveness of LDV-carrying macrophages to the action of interferon on superinfecting VSV*[a]

Macrophages infected with:	Superinfected with:	% CPE (48 h after infection with VSV)
No LDV	VSV	90
No LDV + IF (100 units)[b]	VSV	60
No LDV	Sham	0
6 days LDV	VSV	90
6 days LDV + IF (100 units)	VSV	55
6 days LDV	Sham	0

[a] Preinfection with LDV with an MOI of approximately 10. Superinfected after 6 days with VSV, 10^5 ID_{50}/ml.
[b] Numbers in parentheses give the units of interferon per tube added to the macrophage cultures, 12 h before sham or superinfection with VSV.

TABLE 9. *Responsiveness of LDV-carrying macrophages to the action of interferon on superinfecting Sendai virus*[a]

Macrophages infected with:	Superinfected with:	% Hemadsorption (48 h after infection with Sendai virus)
No LDV	Sendai	100
No LDV + IF (10 units)[b]	Sendai	25
No LDV	Sham	0
6 days LDV	Sendai	100
6 days LDV + IF (10 units)	Sendai	40
6 days LDV	Sham	0

[a] Preinfection with LDV with an MOI of approximately 10. Superinfected after 6 days with Sendai virus, 10^8 ID_{50}/ml.
[b] Numbers in parentheses give the units of interferon per tube added to the macrophage cultures, 12 h before sham or superinfection with Sendai virus.

action of IF, both in vivo and in vitro. Such a combination of IF characteristics indicates that IF does not play a major role during LDV infection. However, enough IF may be produced during the first few days of in vivo infection (Fig. 1) to participate in the early decline of virus titers to the lower levels which persist thereafter. It is not known how the virus level is maintained below its peak between the 72nd hour, when circulating IF is no longer demonstrable, and the 2nd week, when the neutralizing antibody response further reduces the virus level (7). It is possible that the LDV-producing macrophages in the chronically infected mouse begin to limit the amount of LDV produced, since LDV superinfection of such macrophages in vitro resulted in decreased levels of LDV multiplication as compared with macrophages from uninfected mice (Table 4).

Earlier studies of persistent viral infections also indicate that the amount of IF produced and the sensitivity of the virus to IF are important determinants of the degree of the IF effect on the pathogenesis of the infection. For example, evidence has been presented that interferon may play a decisive role in the maintenance of certain persistent tissue culture infections by poliovirus (15, 16), by VSV (14), by vaccinia virus (12), by rubella virus (24), and by rabies virus (23). One case where interferon apparently did not play a role in the persistent infection is the mouse L cell culture, chronically infected with tick-borne encephalitis virus—the infected cultures became unresponsive to interferon (22). No attempts to cure with exogenous interferon were reported in the abovementioned cases. Hallum et al. (13), however, reported such treatment with high concentrations of interferon-cured L cells which were persistently infected with the Herts strain of NDV—NDV was sensitive to interferon in this system. Our study shows that LDV-infected macrophages do not become refractory to the action of exogenous interferon (Tables 7, 8, and 9) as occurs in the

TABLE 10. *Action of repeated IF administrations in vitro starting 12 h before (expt 1) or 12 h after (expt 2) LDV inoculation[a] of macrophage cultures*

Expt	Concn of IF (units/tube)	Virus titers (log_{10} ID_{50}/ml ± SD) of supernatants of macrophage cultures at various times after infection									
		5th wash[b]	12 h	24 h	36 h	2 days	2.5 days	3 days	3.5 days	4.5 days	5 days	6.5 days
1	Control[c]	3.3 ± 0.20	5.9 ± 0.25	7.1 ± 0.32	7.5 ± 0.25	7.3 ± 0.20		6.3 ± 0.20			6.9 ± 0.25	
	1,000	3.1 ± 0.32	3.3 ± 0.32	5.5 ± 0.28	4.1 ± 0.20	4.1 ± 0.20		3.7 ± 0.20			2.3 ± 0.32	
2	Control[d]	2.5 ± 0.20	5.3 ± 0.32		6.1 ± 0.24		5.5 ± 0.28		6.3 ± 0.20	5.9 ± 0.40		7.1 ± 0.28
	500				5.5 ± 0.42		4.3 ± 0.35		3.7 ± 0.20	3.1 ± 0.28		1.1 ± 0.46
	1,000				5.5 ± 0.42		4.7 ± 0.37		4.5 ± 0.28	3.3 ± 0.35		1.9 ± 0.37

[a] Multiplicity of infection with LDV, approximately 10.
[b] 5th wash, Supernatant of macrophages after four washes, 1 h after LDV infection.
[c] Medium or IF replaced every 24 h for 4 days.
[d] Medium of IF replaced every 24 h for 6.5 days.

tick-borne encephalitis model. Further, different from the NDV model, treatment with high concentrations of interferon could reduce the LDV titer but not cure the chronic LDV infection in macrophages culture (Table 10), although the cells were exposed to IF for 5 days after they showed a maximum LDV titer (12 to 18 h after infection (6). These experiments were terminated, because after 6 days the IF-treated cells progressively rounded up and lost adherence, due to a slowly progressive toxicity of the IF preparations used, both in virus-infected sets and in IF control sets in other experiments. This toxicity could have contributed to the decline in virus titer noted (Table 10). This toxicity occurred too late to account for the early inhibition of LDV. Experiments with purified interferon will be necessary to determine the effect of prolonged IF treatment. The inability to cure this model infection during the 6 days of treatment may be due to the relative insensitivity of LDV to interferon. It is not known whether this insensitivity is due to a nonheritable IF-resistant virus fraction (21), to maintenance of the virus at sites protected against IF, or to unknown factors.

Our study further shows that the virus-macrophage cultures provide a model in which the patterns of pathogenicity of the different viruses used are similar in vivo and in vitro, as is the action of IF on these patterns. In the case of VSV, both a 100% lethal infection or a 100% CPE are reduced with increased IF treatment; IF treatment reduces the Sendai virus titer in the lung and the hemadsorption in vitro, and it temporarily reduces both the chronic LDV viremia in the mouse and LDV titer in the tissue culture supernatants. Such a similarity in action both in vivo and in vitro is often not the case (e.g., reference 20). In the case of LDV, this in vivo and in vitro correlation may be due to the involvement of one cell type, the macrophage, as the target cell for LDV infection under both conditions.

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ADDENDUM IN PROOF

S. Yamazaki and A. L. Notkins (J. Virol. **11**:473-478), in contrast to the present findings, report that LDV is as sensitive to interferon in vitro as is the interferon-sensitive vesicular stomatitis virus. This difference might be attributable to differences in the experimental conditions including (i) use of mouse embryo cultures which include many cell types, be-

sides macrophages and (ii) differences in strains of LDV. No in vivo data were reported.

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