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Interferon in Experimental Viral Infections in Mice: Tissue Interferon Levels Resulting from the Virus Infection and from Exogenous Interferon Therapy

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In mice given single intraperitoneal doses of interferon, serum interferon levels peaked at 1 h postinjection and were reduced to zero at about 8 h. The interferon concentrations in spleen, liver, and lungs were about 100-fold higher than could be expected from the amount of serum contained in these organs. In the brain only low levels of antiviral activity were detected. In mice infected intraperitoneally with Mengo virus, viral replication in the brain occurred around day 4 and was accompanied by the appearance of large amounts of interferon ($\sim 10^{3.25}$ U/g). This was preceded, however, by viral replication in the spleen and by the appearance of modest amounts of interferon in spleen and serum. In these mice protection could be obtained with relatively small doses of interferon, provided they were given before the time of maximal levels of endogenous serum interferon. In mice infected intranasally with vesicular stomatitis virus, virus replication in the brain started within 24 to 48 h and increased with time; also, small amounts of interferon (10^2 to $10^{2.5}$ U/g) were already detectable on days 1 and 2. The major peak of virus replication in the brain occurred on days 5 to 6 and was accompanied by the appearance of large amounts of interferon ($\sim 10^{3.25}$ U/g). In this model early treatment with interferon also provided protection, but only if given in larger doses than in the Mengo virus system. Athymic (nu/nu) mice developed a chronic systemic infection when inoculated with a dermotropic strain of vaccinia virus. No interferon was detected in sera, livers, spleens, or lungs of these animals; some mice had low levels of interferon-like antiviral activity in the brain, but no attempt was made to characterize this material. Daily administration of large doses of interferon failed to exert an effect on the development of this chronic disease. Yet, normal (NMRI) mice were protected against acute infection with dermotropic or neurotropic strains of vaccinia virus, and athymic mice were partially protected against acute lethal infection with neurotropic vaccinia virus.

Endogenous interferon plays an important role in the spontaneous cure of certain viral infections (11, 13). Hence, it would be logical to postulate that, for exogenous interferon to be of additional benefit to the host, it should reach the sites of viral replication either earlier than the local burst of interferon production or in concentrations which are significantly higher than those already present as a result of endogenous interferon production. The protective effects of various interferon treatment regimens have been studied in several experimental viral infections in mice (4, 5, 8, 10, 12, 16, 21). Unfortunately, little or no information was provided about the sites, times, and amount of endogenous interferon production in those systems. The present study was undertaken to relate effectiveness or ineffectiveness of interferon treatment to its ability or inability to reach target organs in a concentration which was comparable to or higher than that already present as a result of induction by the challenge virus. We have studied: (i) the organ distribution of exogenously administered interferon in mice, (ii) the kinetics of endogenous interferon production in three experimental viral infections in mice, and (iii) the protective effect of systemically administered interferon in each of these model systems.

MATERIALS AND METHODS

Mice. NMRI mice and athymic homozygous nude (nu/nu) mice of NMRI background were obtained from the "Proedierencentrum" of the University of Leuven. The nu/nu mice were bred according to breeding schedule IV of Giovanella and Stehlin (9). Mice were kept in stainless-steel cages; food and water were provided *ad libitum*.

Viruses. Mengo virus was propagated on L-929 cells and titrated by inoculation of groups of five NMRI mice (21 days old) with serial threefold dilutions (0.2 ml) intraperitoneally. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and

Muench (17). Vesicular stomatitis virus (VSV, Indiana strain) was propagated on L-929 cells, Vero cells, or primary chicken embryo cells derived from 10-day-old embryonated eggs. The LD_{50} titers were determined by intranasal inoculation of 21-day-old NMRI mice. Two strains of vaccinia virus were used: a dermovaccinia virus strain (D-strain) used for vaccination of humans (received from the Belgian State Vaccine Office) and a neurovaccinia virus strain (N-strain) obtained from M. Worthington (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). Stocks of both strains were prepared on the chorioallantoic membrane of 11-day-old chick embryos. The infectivity of vaccinia virus stocks was tested by plaque assay on RK13 cells, primary chicken embryo fibroblasts, or secondary mouse embryo fibroblasts, as indicated in the text. Semliki Forest virus was originally obtained from R. Friedman (National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md.); it was propagated and titrated on Vero cells.

Interferon preparations. Mouse interferon was prepared on mouse L-929 cells with Newcastle disease virus (Komarov strain), concentrated and partially purified by fractional precipitation with ammonium sulfate as described elsewhere (14). The interferon preparations had a specific activity in the range of 10^7 U/mg of protein.

Interferon titrations. The activity of interferon for injection was titrated by the microtiter method of determining inhibition of cytopathic effects as described earlier (14). Interferon activity in sera and tissue homogenates was assayed by measuring the incorporation of [^{3}H]uridine into acid-precipitable material of Semliki Forest virus-infected 5-day-old mouse embryo fibroblast cultures treated with actinomycin D. Mouse embryo fibroblasts were seeded in glass vials 1 cm in diameter (designed for measurements of radioactivity in scintillation counters) at a density of 3×10^4 cells per vial in Eagle minimal essential medium (EMEM) supplemented with 10% heat-inactivated fetal bovine serum (Gibco Biocult, Glasgow, Scotland), 2 mM glutamine, and 2.2 g of sodium bicarbonate per liter. After the cells were grown to confluency, medium was decanted and serial 0.5 $\log_{10}$ dilutions were made in EMEM with 2% FBS were added to duplicate cultures (0.4 ml per vial). In each assay a laboratory mouse interferon standard consisting of mouse L-929 cell interferon induced with Newcastle disease virus was included. After overnight incubation at 37°C, the fluids were decanted and the cultures were challenged with 0.2 ml of EMEM with 2% fetal bovine serum containing Semliki Forest virus (10^7 plaque-forming units [PFU]/ml) and actinomycin D (1 μg/ml). Cell controls received only EMEM with actinomycin D. After 2.5 to 3 h of incubation at 37°C, 0.2 ml of EMEM with 2% fetal bovine serum containing 1 μCi of [^{3}H]-uridine (specific activity, 25 to 29 Ci/mmol, IRE, Mol, Belgium) was added to each culture, and incubation was continued for a further 2.5 to 3 h. Then the fluid was aspirated, and the cells were washed twice with ice-cold 5% trichloroacetic acid and once with ethanol. After thorough drying of the cell sheet, the content of each vial was dissolved in 100 μl of tissue solubilizer (1 part of Soluene-350 and 3 parts of toluene) for 10 min at room temperature. Acid-precipitable radioactivity was counted in each culture in a toluene-based scintillant. Interferon titer was expressed as the dilution of the interferon sample that reduces the net incorporation (observed counts per minute minus "blank" counts per minute of cell control) to 50% of the control (without interferon).

With this assay no antiviral activity was detectable in serum, brain, or lungs of mice that were not infected or not given exogenous interferon. Most spleen and liver homogenates, on the contrary, contained detectable antiviral activity, as indicated in Table 1. The levels were too low to permit characterization of this activity. Therefore, the antiviral activity formed in infected mice or mice treated with exogenous interferon was only considered as interferon when the levels were $>10^{2.2}$ U/g for spleen and $>10^{2.4}$ U/g for liver. All interferon doses in the present study are expressed as international units per milliliter in terms of the National Institutes of Health standard preparation G-002-904-511.

Tissue processing. Blood samples for determinations on serum were taken from the orbital sinus. Brain, spleen, liver, lung, and peripheral lymph nodes were removed at intervals after inoculation and homogenized by a motor-driven Teflon-pestle tissue homogenizer in 9 volumes of EMEM supplemented with 2% fetal bovine serum, 2 mM glutamine, and 2.2 g of sodium bicarbonate per liter.

Infectious virus assays. VSV and vaccinia virus were assayed by plaque formation on Vero cells. Mengo virus was titrated on CCL-23 cells (HEp-2 cells) in flat-bottom microtiter plates (Falcon Plastics, Oxnard, Calif.). Cells were seeded at a density of 6×10^4 cells per well. After overnight incubation at 37°C, serial threefold dilutions of virus samples were added (20 μl per well) to quadruplicate cultures. Plates were incubated at 37°C for 3 days. The cytopathic effect was read microscopically, and the results were calculated as 50% tissue culture infectious doses per milliliter.

RESULTS

Interferon levels in tissues of mice after intraperitoneal injection of homologous interferon. Groups of three to six mice were injected intraperitoneally with $10^{5.0}$ U of interferon. At various time intervals the animals were bled and killed. Spleens, brains, liver, lungs, and inguinal lymph nodes were taken. Figure 1 shows the results of interferon determinations done on the homogenates of these organs. The curve of serum interferon showed a peak of $10^{3.0}$ U/ml at 1 h postinjection and then rapidly declined to virtually zero at 8 h. In the spleen a peak of interferon also occurred at 1 h, but the concentration, in units per gram of wet tissue, was about 10-fold higher than in the serum; also, significant amounts of interferon could still be detected in the spleen at 8 h. A roughly similar pattern was observed with lung and liver. In lymph nodes the interferon concentrations were only slightly higher than in the serum, and brain tissue contained only small amounts of inter-

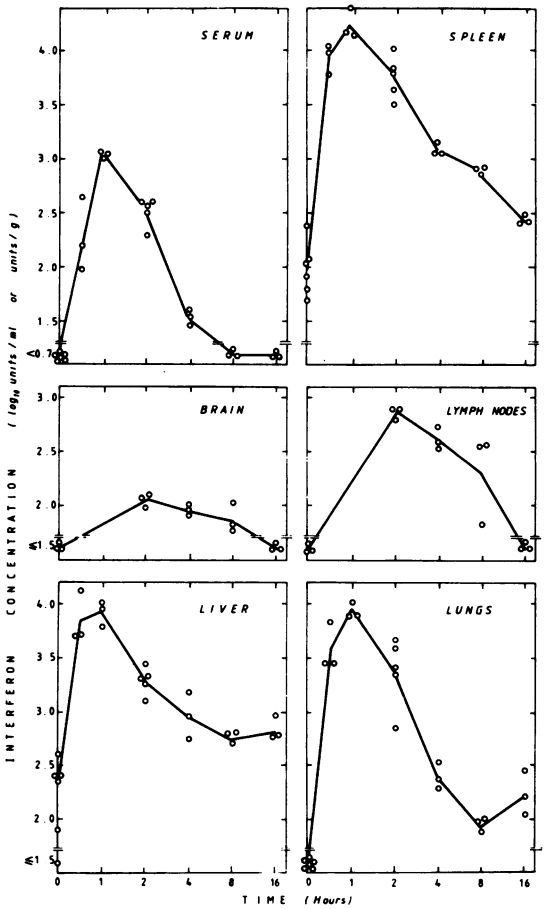

FIG. 1. *Interferon levels in tissues of mice after intraperitoneal injection of 10^5 U of homologous interferon.*

feron at any time.

Comparatively high titers in the tissues may relate to potentiation of interferon by factors extracted during the homogenization process or to the presence of inhibitors of interferon in the serum. A mixing experiment done to test this possibility is shown in Table 1. It can be seen that the presence of both serum and tissue homogenates slightly potentiated the action of interferon in the titration system. Thus, it seems unlikely that the difference in interferon titers between serum and spleens can be accounted for by a different relative content of potentiating or inhibitory factors.

Interferon levels in tissues of mice during experimental viral infections. Lethal mengo virus infection in normal (NMRI) mice was produced by inoculating the mice intraperitoneally with an approximate LD_{90} of an encephalitogenic strain of Mengo virus. At daily intervals, groups of three to six mice were bled and killed. Spleens and brains were taken for interferon determinations. Significant serum titers were found only on days 2, 3, 4, and 5, with a peak value on day 3 (Fig. 2). The spleens contained variable amounts of virus, as shown in Fig. 3. Yet, it can be seen that maximal virus replication occurred about 2 days earlier in the spleen than in the brain, suggesting that the brain was seeded by virus primarily produced in the spleen and possibly some other lymphoid tissues. In the brain there was a good parallelism between interferon titers and virus replication; moreover, the interferon levels were definitely higher than those present in the serum. Since brain tissue does not have the ability to take up interferon from the serum, this interferon must have been produced locally. Spleen interferon was already detectable on the first day of infection, suggesting that this organ was an active producer. However, some of the interferon found in the spleen

TABLE 1. *Influence of serum and tissue homogenates on mouse interferon titrations*

Final concn (log_{10} U/ml) of interferon added to mixture	Titer (log_{10} U/ml) obtained when interferon was mixed (vol/vol) with:			
	Saline	Mouse serum	Spleen homogenate[a]	Brain homogenate[a]
3.3	3.4	3.7	3.6	3.7
4.3	4.3	4.9	4.6	4.6

[a] Tissue homogenates in 10 volumes of saline prepared as described in Materials and Methods.

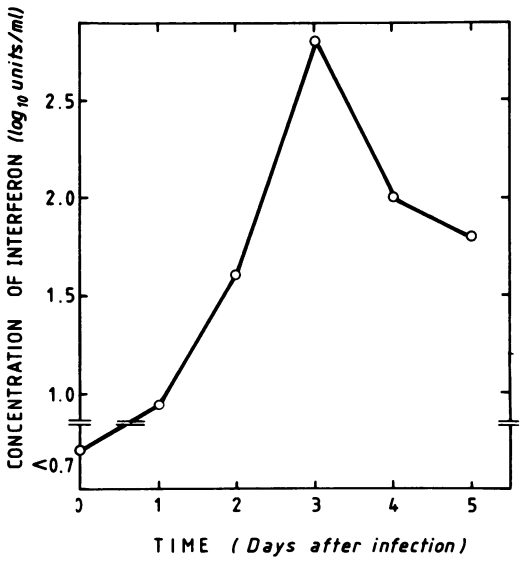

FIG. 2. *Interferon levels in sera of mice infected with $10^{3.5}$ PFU (= 1 LD_{90}) of Mengo virus intraperitoneally. Each time point represents an average of values obtained for three to six mice.*

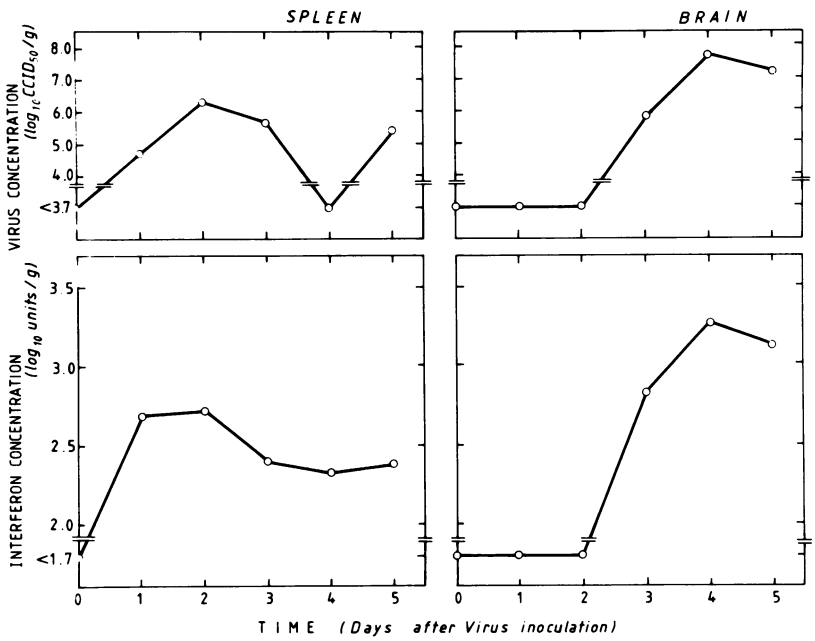

FIG. 3. *Interferon and virus concentrations in tissues of mice infected with $10^{3.5}$ PFU (= 1 LD_{90}) of Mengo virus intraperitoneally. Each time point represents the average of values obtained for three mice.*

may also have come from other organs. It is of particular interest to compare the levels of endogenous tissue interferon with those obtained after the administration of a high dose of exogenous interferon. It is clear that exogenous administration could easily bring about spleen levels that were comparable to those during Mengo virus infection. In contrast, brain levels obtained after exogenous administration of a high dose of interferon were inferior to those generated by local production. Hence, one can predict that the administration of interferon early during infection can have a beneficial influence, whereas late administration (e.g., starting on day 3 postinfection), when virus is already actively replicating in the brain, will be much less effective, if effective at all.

Intranasal inoculation of mice directly infects the central nervous system though the olfactory nerve (19). To study endogenous interferon production in this system, we inoculated 21-day-old NMRI mice intranasally with $10^{5.0}$ PFU (~1 LD_{90}) of VSV. At daily intervals the brains of three to six mice were collected for determination of infectious virus and interferon content. The results of two experiments, done with different preparations of VSV, are shown in Fig. 4. Virus replication became detectable on day 1 or 2 and then progressively increased to reach a peak level between days 4 and 6. Some of the mice analyzed on day 5, 6, and 7 were, in fact, moribund. Interferon activity (100 to 300 U/g of tissue) was detectable in the brains of most mice killed on days 1 and 2. Thus, endogenous interferon production started early during infection. During subsequent days interferon titers in the brain paralleled virus replication and reached levels of 10^3 to $10^{3.5}$ U/g. From this pattern one may predict that systemic administration of interferon may provide protection in this system only when given early and in high doses so as to bring about brain levels of more than 100 to 300 U/g of tissue.

We also studied endogenous interferon production in a chronic virus infection. It was found that nu/nu mice infected intravenously with a dermotropic strain of vaccinia virus developed cutaneous lesions which persisted for several weeks. Infectious virus could always be isolated from these skin lesions ($10^{5.5}$ to $10^{7.0}$ PFU/g). To study endogenous interferon production, we inoculated 25-day-old nu/nu mice intravenously with $10^{4.8}$ PFU of vaccinia virus (D-strain). Groups of 3 to 4 mice were killed after 1, 2, 3, and 4 weeks, and different organs were taken for determination of infectious virus as well as interferon content (Table 2). Lung tissue contained around $10^{3.3}$ PFU per g of tissue in virtually all mice at all times. In brain, spleen, and liver homogenates virus was sporadically detectable by the plaque assay used in our study. Finally, significant viremia could be demonstrated in the majority of mice killed after 3 to 4 weeks but not in those killed earlier. No sig-

nificant interferon activity could be detected in the sera, lungs, spleens, or livers of the infected mice. The brain homogenates of some mice contained levels of virus-inhibitory activity, which were too low to permit characterization (equivalent of $\sim 10^{2.0}$ U of interferon per g of tissue). Therefore, the chronic dermovaccinia virus infection of nu/nu mice was considered as essentially non-interferonogenic. From this, one might predict that exogenous interferon has a chance to affect the course of this chronic disease.

Comparative sensitivities of VSV, Mengo virus, and vaccinia virus to the in vitro antiviral effect of interferon in mouse cells. The three viruses used for in vivo protection studies were tested for sensitivity to the in vitro antiviral effect of interferon in mouse cells. This was done by a simple single-cycle yield reduction assay on first-passage NMRI mouse embryo fibroblasts. The insensitivity of vaccinia virus (N-strain) is illustrated in Table 3. It can be seen that, under the conditions used, VSV responded to $10^{0.2}$ U/ml and Mengo virus to $10^{1.2}$ U/ml. With the N-strain of vaccinia virus the 0.5 $\log_{10}$ reduction point ranged between $10^{2.5}$ and $10^{3.5}$ U/ml. Even with as much as $10^{3.7}$ U/ml, vaccinia virus replication was only inhibited by a factor of $10^{1.1}$ while VSV and Mengo virus replication was reduced by factors of $10^{3.6}$ and $10^{2.5}$, respectively. The in vitro sensitivity of the D-strain could not be tested as this virus failed to replicate in mouse cells in vitro.

Effect of exogenous interferon on the evolution of experimental virus infections. The predictions made in the previous sections were tested by treating mice with Mengo virus, VSV, or dermovaccinia virus with exogenous interferon.

Lethal Mengo virus infection was produced in normal (NMRI) mice by inoculating groups of 10 mice (21 days old) intraperitoneally with Mengo virus, $10^{3.5}$ PFU (~ 1 LD_{90}) or 10^5 PFU (>1 LD_{100}), on day 0. Interferon was given intraperitoneally at dosages of 0, 10^4, and 10^5 U. In the first part of the experiment, the injections were given on days 0, 1, 3, 5, and 7 postinfection (early treatment). In the second part, the injections were given daily from days 3 to 11 (late treatment). When interferon treatment was started early, a protective effect was seen with both dose levels against both challenge doses (Fig. 5A and B). With treatment started late, no protection could be obtained against the higher virus dose (Fig. 5C), and significant protection against a low virus challenge was only seen with the highest interferon dose (Fig. 5D). Thus, these experiments bore out the prediction made from the study of endogenous interferon production and virus replication.

To test the effect of exogenous interferon on lethal VSV infection in normal (NMRI) mice,

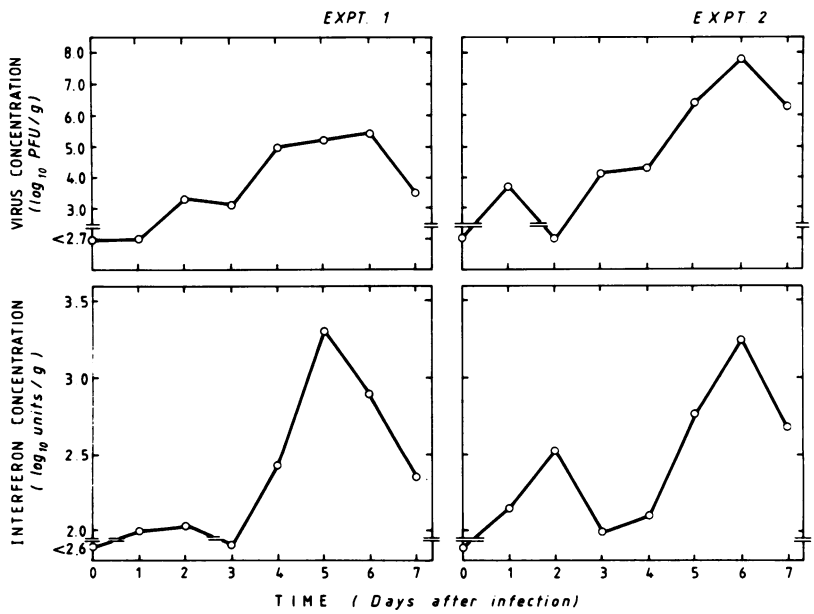

FIG. 4. *Interferon and virus concentrations in the brains of mice infected with $10^{5.0}$ PFU on VSV intranasally. Experiment 1: VSV propagated in L-929 cells. Experiment 2: VSV propagated in Vero cells. Each time point represents an average of values obtained for three to six mice.*

TABLE 2. *Detectability of virus and interferon in blood and organs of nu/nu mice chronically infected with vaccinia virus (D-strain)*

Day	Mouse no.	Virus[a]					Interferon[b]				
		Serum	Brain	Spleen	Liver	Lung	Serum	Brain	Spleen	Liver	Lung
0	1						−	−	−	−	−
	2			Not determined			−	−	−	−	−
	3						−	−	−	−	+
6	4	−	−	−	−	+	−	−	−	−	−
	5	−	−	−	−	+	−	−	−	−	−
	6	−	+	−	−	+	−	−	−	−	−
	7	−	−	−	−	+	−	+	−	−	−
14	8	−	−	−	−	+	−	−	−	−	−
	9	+	−	−	−	+	−	−	−	−	−
	10	−	−	−	−	+	−	−	−	−	−
	11	−	−	−	−	−	−	+	−	−	−
21	12	+	−	−	−	+	−	+	−	−	−
	13	−	−	−	−	+	−	−	−	−	−
	14	+	−	+	−	−	−	+	−	−	−
	15	−	−	+	+	−	−	−	−	−	−
28	16	+	−	−	−	+	−	−	−	−	−
	17	+	−	−	−	−	−	+	−	−	−
	18	−	+	−	+	+	−	−	−	−	−
	19	+	−	−	−	+	−	−	−	−	−
Avg value[c] of positives		2.7	3.1	2.8	3.2	3.3		2.0			1.9

[a] Symbols: +, $\geq 10^{2.2}$ PFU/ml of serum or $\geq 10^{2.7}$ PFU/g of wet tissue; −, $<10^{2.2}$ PFU/ml of serum or $<10^{2.7}$ PFU/g of wet tissue.

[b] Symbols: +, >base levels in organs of control mice; −, ≤base levels in organs of control mice. Base levels were as follows: serum, $<10^{1.3}$ U/ml; brain and lung, $<10^{1.5}$ U/g; spleen, $10^{1.2}$ to $10^{2.4}$ (98% confidence limits) U/g; liver, $10^{1.4}$ to $10^{2.4}$ (98% confidence limits) U/g.

[c] Virus titer: $\log_{10}$ PFU per milliliter of serum or per gram of wet tissue. Interferon-like antiviral activity: $\log_{10}$ units per milliliter or per gram of wet tissue.

TABLE 3. *Sensitivity of VSV, Mengo virus, and vaccinia virus (N-strain) to the antiviral effect of mouse interferon in primary mouse embryo fibroblasts*

Interferon concn ($\log_{10}$ U/ml)	VSV	Mengo virus	Vaccinia virus (N-strain)
0.2	1.0[a]	<0.5	<0.5
1.2	2.1	0.5	<0.5
3.7	3.6	2.5	1.1

[a] Reduction in virus yield ($\Delta \log_{10}$ PFU per milliliter). Virus yields (PFU per milliliter) in cells not treated with interferon were $10^{7.0}$ for VSV, $10^{5.8}$ for Mengo virus, and $10^{6.4}$ for vaccinia virus.

we inoculated groups of 10 mice (21 days old) on day 0 with an estimated LD_{90} of VSV ($\sim 10^{5.0}$ PFU) intranasally. Interferon was given intraperitoneally at dosages of 0, 10^4, and 10^5 U on days −1, 1, 3, 5, and 7. Table 4 shows the results of two experiments. It can be seen that protection was minimal or nonexistent with doses of 10^4 U but was significant with a 10-fold higher dosage. Thus, the intranasal VSV system appeared much less sensitive to a standard treatment protocol than the Mengo virus system, although VSV was considerably more sensitive to interferon in vitro. This is also in agreement with the prediction made from the study of endogenous interferon production and virus replication.

Exogenous interferon was also tested against chronic disease in athymic (nu/nu) mice infected with dermotropic vaccinia virus. Three groups of 10 nu/nu mice were infected intravenously with $10^{4.8}$ PFU of vaccinia virus (D-strain) on day 0. The first group (control) received no additional treatment; the second group (early treatment) received $10^{4.7}$ U of interferon daily from days −1 to 5; and the third group (late treatment) received daily injections from days 11 to 46 in an attempt to cure existing cutaneous lesions and to prolong the life-span of the mice. Figure 6 shows a slight delay in the mortality

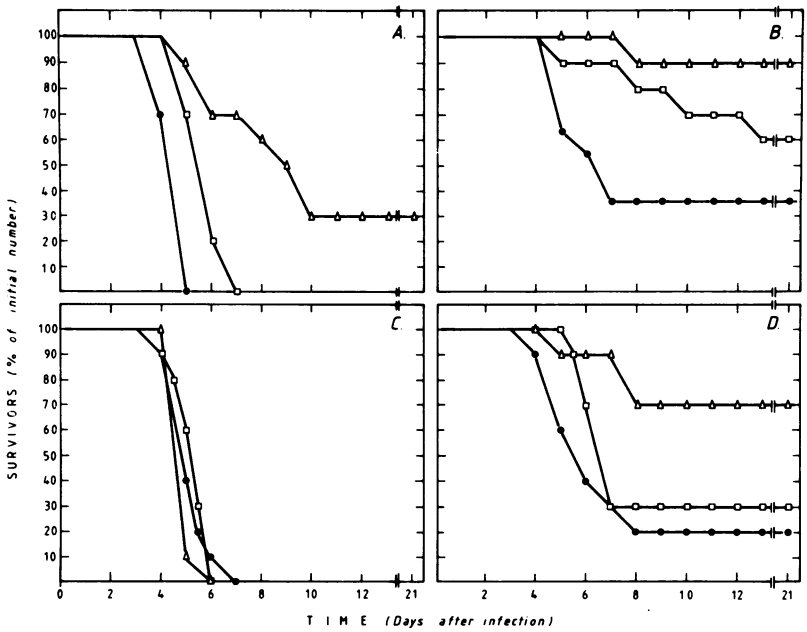

FIG. 5. *Protection of NMRI mice by interferon against Mengo virus infection. Left panels (A and C): high virus dose (10^5 PFU per mouse, intraperitoneally). Right panels (B and D): low virus dose ($10^{3.5}$ PFU per mouse, intraperitoneally). Upper panels (A and B): early treatment with interferon (intraperitoneal injections on days 0, 1, 3, 5, and 7 postinfection). Lower panels (C and D): late treatment with interferon (daily intraperitoneal injections from days 3 to 11). Symbols: △, 10^5 U of interferon per injection; □, 10^4 U of interferon per injection; ●, saline injections.*

TABLE 4. *Interferon protection in mice after intranasal infection with VSV*

Treatment	No. of survivors/total		P[a]	
	Expt 1	Expt 2		
Phosphate-buffered saline	3/11	1/10	>0.8	<0.005
Interferon, 10,000 U per mouse	7/10	1/10		
Interferon, 100,000 U per mouse	9/10	4/10		

[a] Chi-square test on pooled data of the two experiments.

curve of mice treated early. However, these mice, as well as the control mice, became chronically infected, as evidenced by the presence of multiple skin lesions and by the fact that the later part of the mortality curve was not different from that of controls. Mice given daily injections of interferon starting on day 11 postinfection also developed chronic disease and died at the same rate as control mice. Thus, daily interferon treatment seemed unable to affect chronic vaccinia infection when started after the appearance of symptoms.

Table 5 shows the effect of exogenous interferon on acute vaccinia virus (N-strain) infection in normal (NMRI) and athymic (nu/nu) mice. Groups of 10 NMRI mice (21 days old) and groups of 6 nu/nu mice (25 days old) were infected intraperitoneally with different doses of neurotropic vaccinia virus (N-strain). They were also given interferon intraperitoneally ($10^{4.7}$ U daily from days −1 to 5 after infection). Mortality was followed. Interferon had a good protective effect in NMRI mice in that it increased the proportion of mice that survived the infection. In athymic mice the infection was lethal for all mice. However, in mice treated with interferon the mean survival time was prolonged by a number of days depending on the dose of virus. These experiments show that interferon can exert a certain protective effect on vaccinia virus infection in vivo, despite the fact that this virus is largely insensitive to the antiviral effect of interferon in mouse cells in vitro.

DISCUSSION

When mice were given single large doses of interferon intraperitoneally, serum interferon levels peaked at about 1 h postinjection and were reduced to virtually zero after 8 h. The interferon titers in spleen, liver, and lungs, expressed in units per gram, were more than 10-fold higher. Mixing experiments made it seem

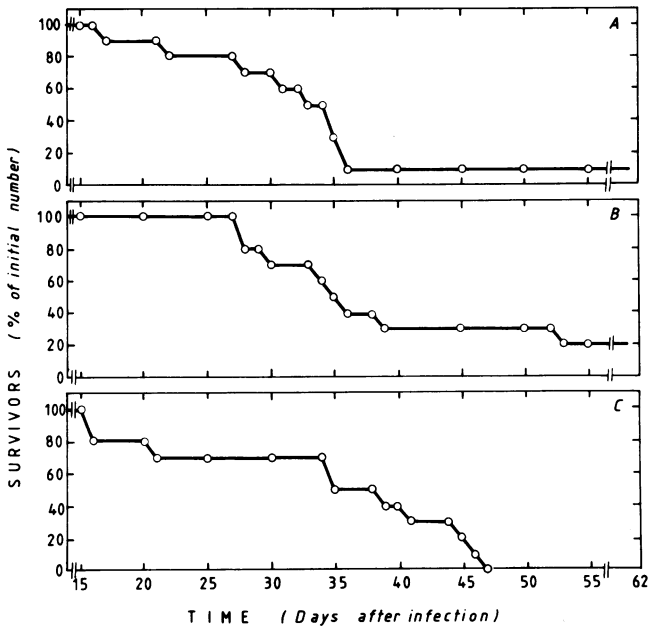

FIG. 6. *Protection of nu/nu mice by interferon against intravenous vaccinia virus (D-strain) injection ($10^{4.8}$ PFU per mouse). (A) Untreated. (B) Daily intraperitoneal injections of interferon ($10^{4.7}$ U) from days −1 to 5 postinfection. Statistical significance of difference with A: $P > 0.1$ (logrank test for survival data of Peto and Pike [17]). (C) Daily intraperitoneal injections of interferon ($10^{4.7}$ U) from days 11 to 46 postinfection. Significance of difference with A: $P > 0.3$.*

TABLE 5. *Protection of mice by interferon against intraperitoneal vaccinia virus (N-strain) infection*

Mouse strain	Dose of vaccinia virus (log_{10} PFU/mouse)	Interferon treatment[a]	% Survival on day 21 postinfection[b]	50% Survival time (days)	P[c]
NMRI	6.5	−	41.6	6.0	
	6.5	+	83.3	>20.0	<0.001
	5.5	−	75	>20.0	
	5.5	+	100	>20.0	
nu/nu	7.3	−	0	5	
	7.3	+	0	5.5	
	6.3	−	0	9.5	<0.02
	6.3	+	0	12.0	
	5.3	−	0	12.0	
	5.3	+	0	17.5	

[a] Interferon ($10^{4.7}$ U) was given intraperitoneally on days −1 to 5 postinfection.

[b] Percentage of initial number (NMRI mice, 10 mice per group; nu/nu mice, 6 mice per group).

[c] Logrank test for survival data of Peto and Pike (17).

unlikely that this difference was due to the presence of different amounts of potentiating or inhibitory factors. Another possible explanation for high organ titers would be that the injected interferon contained an interferon inducer, e.g., virus or endotoxin. This possibility cannot at present be excluded but seems unlikely since mice injected with mock interferon failed to show tissue levels. We therefore favor the interpretation that the spleen, liver, and lungs actively took up interferon from the circulation. Such uptake of interferon by cells and subsequent release as a biologically active moiety after homogenization has been shown in vitro (22). In contrast to liver, spleen, and lungs, the brains contained only very small amounts of interferon activity. One may, therefore, expect that exogenous interferon may be especially active on viral events taking place in the spleen, liver, and lungs, and less so on viral replication in the brain. High concentrations of interferon in spleen and liver have been reported in rats and rabbits given high doses of inducer viruses (6, 15). This has mostly been interpreted as indicating that these organs were actively producing interferon. While this still remains a likely possibility, our finding indicates that some of the endogenous interferon found in spleens, livers, and lungs of infected animals may in fact have been produced elsewhere, e.g., by circulating lymphocytes. Another corollary of our finding is that high levels of serum interferon may not always be an index for significant protection to occur in organs: this is an important issue in

the clinical application of interferon in humans, as it has been found that lower levels of serum interferon are found with certain types of interferon than with others (1). In view of our findings, such interferons need not necessarily be less active on organ systems.

Endogenous interferon produced during experimental viral infections is in large part responsible for eventual spontaneous cures (11, 13). From this it may be postulated that, for added exogenous interferon to exert a favorable effect, it should reach the sites of viral involvement either earlier or in much higher concentrations than the endogenous interferon produced by the viral infection itself. In mice infected intraperitoneally with a lethal dose of Mengo virus, viral replication was detectable first in the spleen and later on in the brain. Interferon also started to appear early in the spleen. Peak levels in the serum, which reasonably reflect overall interferon production in the body, peaked on day 3, still in time to reach the brain before the major burst of virus replication in that organ. Apparently, this interferon was unable to protect the mice, as was also the large amount of interferon produced by the brain during local virus replication. From this it was inferred that, in this infection, only the interferon generated or given before day 3 could exert any effect on the outcome of the infection. This prediction was, in fact, borne out by the in vivo protection experiments: exogenous interferon did protect the mice if the treatment was started before virus infection, but not if started on day 3 postinfection. The dose of interferon needed to obtain this protection was relatively small, conceivably because of active uptake by organs, such as the spleen, where primary virus replication took place.

In mice infected intranasally with VSV, virus replication in the brain started rapidly (day 1 or 2) and increased with time. Small amounts of interferon (<100 U/g) were detectable during the first few days; larger amounts (~100 U/g) appeared from day 4 until death. From this it was predicted that exogenous interferon might provide some protection if given in doses high enough to establish levels in the brain of at least $10^{2.0}$ to $10^{2.5}$ U/g. In actuality, this prediction was borne out by the protection experiments: only when doses of 10^5 U per mouse were given could some protection be obtained. It is not clear why in earlier studies (5) protection could be obtained with much smaller doses. Age and species of the animals and virus strain seemed not to be involved; differences in the interferon preparation however, could not be tested.

We also studied the relation between endogenous interferon production and protection by exogenous interferon in mice infected with vaccinia virus. As a model system we chose athymic mice infected with dermotropic vaccinia virus by the intravenous route. Such mice developed a chronic systemic infection. Of all organs tested the skin and lungs contained the highest virus titers at all times; lower titers were also found at one time or another in brain, lymph nodes, spleen, and liver. As interferon remained undetectable, with the possible exception of occasional low levels in brain, we expected exogenous interferon given at a late time during infection to be able to cure the chronic disease. This expectation was not fulfilled in protection experiments. The interpretation of this finding is complicated by the fact that vaccinia virus seemed to be highly insensitive to the in vitro antiviral effect of some interferons: this was found to be the case for human (2) and monkey interferon (20) for the neurotropic strain of vaccinia virus used in our study. It could not be verified for the dermotropic strain which failed to replicate in mouse cells in vitro. Despite this insensitivity, interferon can protect mice against acute vaccinia virus infection. Intravenous infection of normal mice with the dermotropic strain is self-limited: it results in skin lesions on the tail which spontaneously disappear. Treatment with interferon was able to reduce the number of pox lesions (3). In our study interferon was also found to protect normal as well as athymic mice against neurotropic vaccinia virus. Therefore, it is not clear why interferon treatment did not affect chronic dermovaccinia virus infection in athymic mice. Various explanations may be conceived. Perhaps the dermotropic strain of vaccinia virus is completely resistant to the direct antiviral effect of interferon, so that only the activation of other host-defense mechanisms, e.g., thymus-dependent immunity (7), are responsible for the interferon-mediated protection seen in normal mice. Athymic mice would in that case not be protected by interferon.

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