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Protective effect of recombinant murine interferon beta against mouse hepatitis virus infection

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Summary

Recombinant murine interferon beta (rMuIFN-β) protected susceptible C57BL/6 mice against lethal mouse hepatitis virus (MHV) infection. rMuIFN-β was life saving if it was given intraperitoneally beginning 21 h before infection and daily thereafter for 9 days, and lengthened the survival time if given from 3 h after infection. rMuIFN-β treatment beginning 24 h after infection was ineffective. The survival rate was dose-dependent, and the 50% effective dose of rMuIFN-β for survival was 1780 IU per day. rMuIFN-β pretreatment inhibited virus growth completely in the brain and moderately in the liver and spleen and prevented severe hepatic lesions. rMuIFN-β also protected beige mice and cyclophosphamide-treated mice against MHV infection, suggesting that activation of natural killer cells or T-cells by rMuIFN-β is not critical for protection.

Interferon; Mouse hepatitis virus; Natural killer cell

Introduction

Interferons (IFNs) have a multitude of diverse biological effects (Kirchner, 1986). There are three antigenically and chemically distinct types of IFNs (known as α,

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β and γ). Since recombinant DNA technology has been applied to the production of the IFNs, large amounts of cloned IFNs are available (Joklik, 1985). Therefore, careful determination of the relative potency of each type or subtype for each of the effects of IFNs now constitutes an important priority (White and Fenner, 1986).

Although simulation experiments with mice may lead to better concepts of treatment or prevention of viral diseases with IFNs, human recombinant IFN-β is of little use in animal experiments because of the host species specificity. Natural murine IFNs are available, but because of the limited amounts and the contamination by IFN-α (Kirchner, 1986), the precise roles of murine IFN-β in the antiviral or immunomodulatory effects have not been clarified.

In this investigation we have studied the effect of new authentic recombinant murine IFN-β (rMuIFN-β) (Matsuda et al., 1986) against mouse hepatitis virus (MHV) infection. MHV, a murine coronavirus, causes hepatitis and/or encephalomyelitis in susceptible mice (Siddell et al., 1983). The strains of MHV include MHV type 1 (MHV-1), MHV-2, MHV-3, MHV-JHM and MHV-D (Siddell et al., 1983; Ishida et al., 1984). The differences in tissue tropisms and disease-producing potentials are in part dependent on the virus strain. MHV-2 Princeton strain is highly virulent for susceptible mice, causing fulminant hepatitis, and is insensitive to low doses of IFNs in vitro (Taguchi and Siddell, 1985). Antibodies (Nakanaga et al., 1983; Nakanaga et al., 1986), macrophages (Bang and Warwick, 1960; Taguchi et al., 1976; Virelizier and Allison, 1976), natural killer (NK) cells (Bukowski et al., 1983), and T cells (Dupuy et al., 1975) are involved in the host response to MHV infection.

Evidence that IFNs can be instrumental in determining the fate of the mouse following MHV infection has been provided by Virelizier and Gresser (1978), who showed that mice infected with sublethal doses of MHV-3 die if anti-IFN serum is administered. Kato et al. (1986) showed that administration of natural murine IFN-α/β significantly prolonged the survival of MHV-2-infected mice. Ishida et al. (1984) showed that MHV killed mice of several strains with low IFN production (e.g., C57BL/6) while it was not pathogenic for most of the other strains of mice, which had significant IFN production (e.g., C3H/He). Therefore, MHV should be suitable as the challenge virus.

In this paper we show the potent antiviral effect of rMuIFN-β against lethal MHV-2 infection. rMuIFN-β not only prolonged the survival time but also completely protected the infected mice from death. We further studied the effects of rMuIFN-β on MHV-infected immunodeficient mice (beige mice and cyclophosphamide-treated mice) and tried to determine the protective mechanism of rMuIFN-β agianst MHV infection.

Materials and Methods

Mice

Five-week-old C57BL/6 mice and C57BL/6 beige (bg/bg) mice of either sex were obtained from the specific-pathogen-free (SPF) colony of our university. The mice

were monitored for antibodies to MHV by the complement fixation test (Wada et al., 1981). The animals were kept in a hood during the experiment and given water and pellet ad libitum.

Cells and viruses

DBT, a Rous sarcoma virus-induced brain tumor cell line, (Kumanishi, 1967) and MHV-2 were originally supplied by Prof. K. Fujiwara and Dr. F. Taguchi (Department of Animal Pathology, Institute of Medical Science, University of Tokyo, Japan) and have been maintained in our laboratories. DBT cells were grown in Eagle's minimal essential medium (MEM) containing 5% calf serum and 5% tryptose phosphate broth. C57BL/6 mice were injected with 5 ml of phosphate buffered saline (PBS, pH 7.2) intraperitoneally (i.p.) and the peritoneal exudate cells (PEC) were collected and grown in MEM containing 5% fetal bovine serum for 3 days. MHV-2 Princeton strain was grown in DBT cells. Infected cells were harvested 48 h after inoculation, sonicated and centrifuged at 2000 rpm for 15 min and the supernatant was stored at −70°C. The virus stock contained 1.7×10^7 plaque-forming unit (PFU)/ml.

The stock virus was diluted with PBS just before use, and each mouse was inoculated i.p. with 100 PFU per 0.1 ml (more than 10 LD_{50}). Mice were observed for more than 14 days after infection. Student's *t*-test was used for statistical analysis.

Vesicular stomatitis virus (VSV) New Jersey strain, grown in DBT cells, was used for in vitro interferon assays.

Interferon

rMuIFN-β, 1×10^7 IU/ml (Matsuda et al., 1986) was supplied by Basic Research Laboratory, Toray Industries, Inc. (Kamakura, Japan). rMuIFN-β undiluted or diluted with 0.03% HCO-60 in 0.15 M NaCl (pH 4.5) was injected i.p. in 0.08-ml quantities into mice every 24 h for 8 or 9 days (for details, see Results).

Cyclophosphamide

Cyclophosphamide (CY) (Endoxan, Shionogi, Osaka, Japan) was dissolved in sterile PBS immediately before use. Mice were injected i.p. with 200 mg of CY per kg 3 days before MHV infection.

In vitro IFN assay

To determine the in vitro sensitivity of MHV-2 to rMuIFN-β, the preparation was assayed by a plaque-reduction method similar to that described by Wagner (1961). Monolayer cultures of DBT cells and PEC in 6-well plates were incubated with 2 ml of 10-fold dilutions of the IFN preparation for 18 h and washed with growth medium, and 100 PFU of VSV or MHV-2 were added to IFN-treated and control cultures. After adsorption at 37°C for 1 h, the cells were overlaid with MEM containing 2% calf serum and 2% methylcellulose, incubated for 4 days at 37°C in 5% CO_2, fixed with 10% formalin and stained with 0.5% crystal violet. The IFN titer is expressed as the 50% plaque-depressing dose (PDD_{50}) (Stewart et al., 1969).

Tissue virus titration and pathology

At various times after virus inoculation, mice were killed for determining the virus titers of their tissues (liver, spleen, and brain). To avoid possible interference by residual rMuIFN-β in the plaque assay, the final rMuIFN-β injection was omitted except for the mice killed 8 h after infection. The samples were weighed and frozen at −70°C until assayed. Each sample was homogenized with a Teflon homogenizer, and after centrifugation at 2000 rpm for 10 min, serial 10-fold dilutions of the supernatant fluid were made with PBS containing 50 μg of gentamicin per ml, and a plaque assay was performed on DBT cell monolayers in 12-well plates. The titers of virus recovered were expressed as PFU per 0.2 g of tissue.

About one-fourth of each liver was fixed immediately after sampling with 10% phosphate buffered formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin for microscopic examination. The degree of liver histopathology was scored as follows: 0 = normal; 0.5 = very slight (small aggregation of inflammatory cells); 1 = slight (a few necrotic foci); 2 = moderate (multiple necrotic foci); 3 = severe (massive necrosis); and 4 = very severe (diffuse necrosis with massive hemorrhage).

Serology

Sera were collected at the time of killing and tested for virus neutralizing activity against MHV-2 by a plaque reduction assay which was essentially the same as described by Taguchi et al. (1976).

Results

Sensitivity of MHV-2 to rMuIFN-β

The sensitivity of MHV-2 to the rMuIFN-β preparation was compared with that of VSV. The PDD_{50} of rMuIFN-β against MHV-2 was 1.1×10^4 IU/ml whereas that against VSV was 5.0 IU/ml. The PDD_{50} of rMuIFN-β against MHV-2 in PEC was 27 IU/ml.

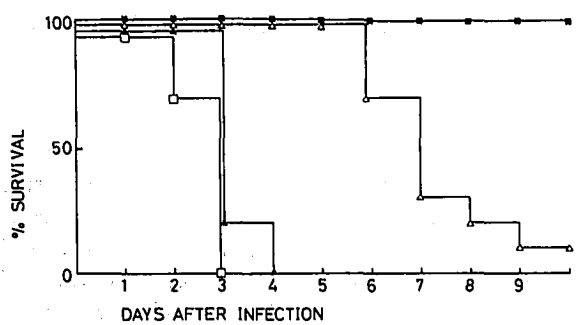

Fig. 1. Effect of rMuIFN-β against MHV infection. Mice were injected with 100 PFU of MHV-2 and 8×10^5 IU of rMuIFN-β was injected beginning 21 h before (■), 3 h after (△) or 24 h after (▲) infection. Control mice (□) were injected with diluent. Each group consisted of 10 mice.

TABLE 1

Dose-dependent effect of rMuIFN-β on MHV-2 infection[a].

rMuIFN-β (IU/day)	Mortality		Survival time (mean days ± SD)
	No.	%	
8×10^5	0/10	0	–
8×10^4	1/6	17	4
8×10^3	0/6	0	–
8×10^2	4/6	67	7.0 ± 1.6
8×10	5/5	100	5.6 ± 1.2
Control	6/6	100	2.7 ± 0.7

[a] Mice were inoculated i.p. with 100 PFU of MHV-2, and were injected i.p. with different doses of rMuIFN-β or with diluent (control) every 24 h beginning 21 h before MHV inoculation.

Effect of time of the initial rMuIFN-β injection

Fig. 1 shows the survival curve of MHV-infected and rMuIFN-β-(or diluent-)-treated mice. Each group consisted of 10 mice. rMuIFN-β treatment was started 21 h before (−21 h), 3 h after (+3 h) or 24 h after (+24 h) infection and continued until 7 days after infection. Control mice were all dead by 3 days after infection (mean survival time: 2.7 ± 0.5 days). All mice of the −21 h group survived 14 days after MHV infection. The survival time of the + 3 h group (mean survival time: 7.0 ± 0.9 days) was significantly longer than that of the control group ($P<0.0005$), but only one of the 10 mice lived until 14 days after infection. Mice of the +24 h group did not survive any longer than the control mice.

Effective dose of rMuIFN-β

The MHV-infected mice were treated with various doses of rMuIFN-β beginning 21 h before infection and continuing for 9 days (Table 1). All but one mouse treated with 8000 IU/day or higher doses of rMuIFN-β survived until 14 days after infection. The 50% effective dose for survival was calculated as 1780 IU/day. Even the mice given 80 IU of rMuIFN-β per day survived significantly longer than the control mice ($P<0.005$).

Neutralizing antibodies were not detected in rMuIFN-β-treated and surviving mice (NT: lower than 10).

Effect of rMuIFN-β on virus growth

Fig. 2 shows the virus growth in the livers, spleens and brains of mice treated with rMuIFN-β or diluent. In the control mice, the virus was first detected on day 1 after infection in the liver and spleen, and on day 2 after infection in the brain, and the titers rose thereafter until death on day 3 after infection. Tissue samples from one group of the rMuIFN-β-treated mice (−21 h) showed a striking reduction in the virus titer; the virus was not detected in the brain throughout the examination, and was detected only on day 3 after infection in the liver and spleen. The virus titers of the livers and spleens of the other group of rMuIFN-β-treated mice (+3 h) were about the same as those of the control group through day 2, but

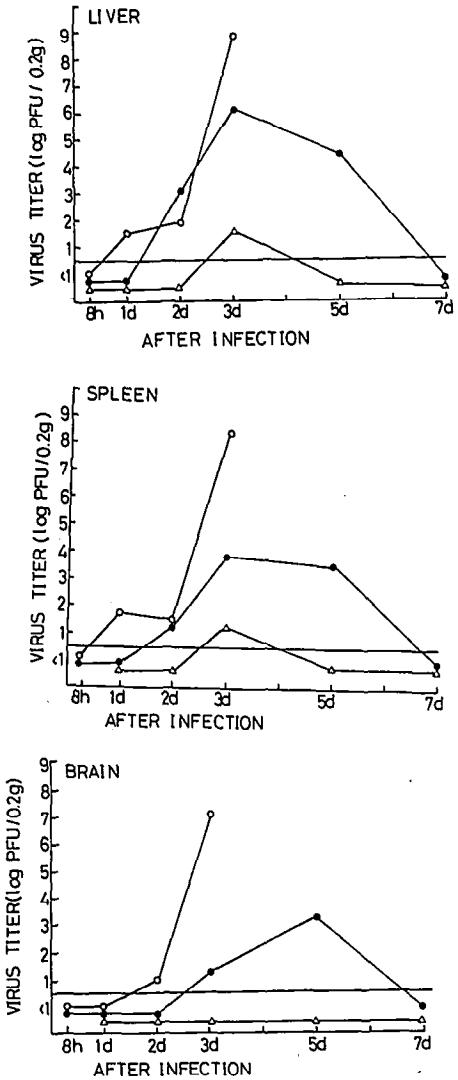

Fig. 2. Virus growth in the liver, spleen, and brain of mice infected with 100 PFU of MHV-2 and treated with rMuIFN-β from 21 h before infection (△) or 3 h after infection (•) and diluent-treated controls (○). rMuIFN-β (8×10^5 IU) was given every 24 h until 7 days after infection. Each point represents the average titer of the tissues of two or three mice.

in the surviving mice, the titers declined from day 5 after infection and virus was not detected on day 7.

Histopathology of liver lesions

The control group showed moderate to severe lesions (massive or diffuse necrosis with hemorrhage and mononuclear cell and polymorphonuclear cell infiltra-

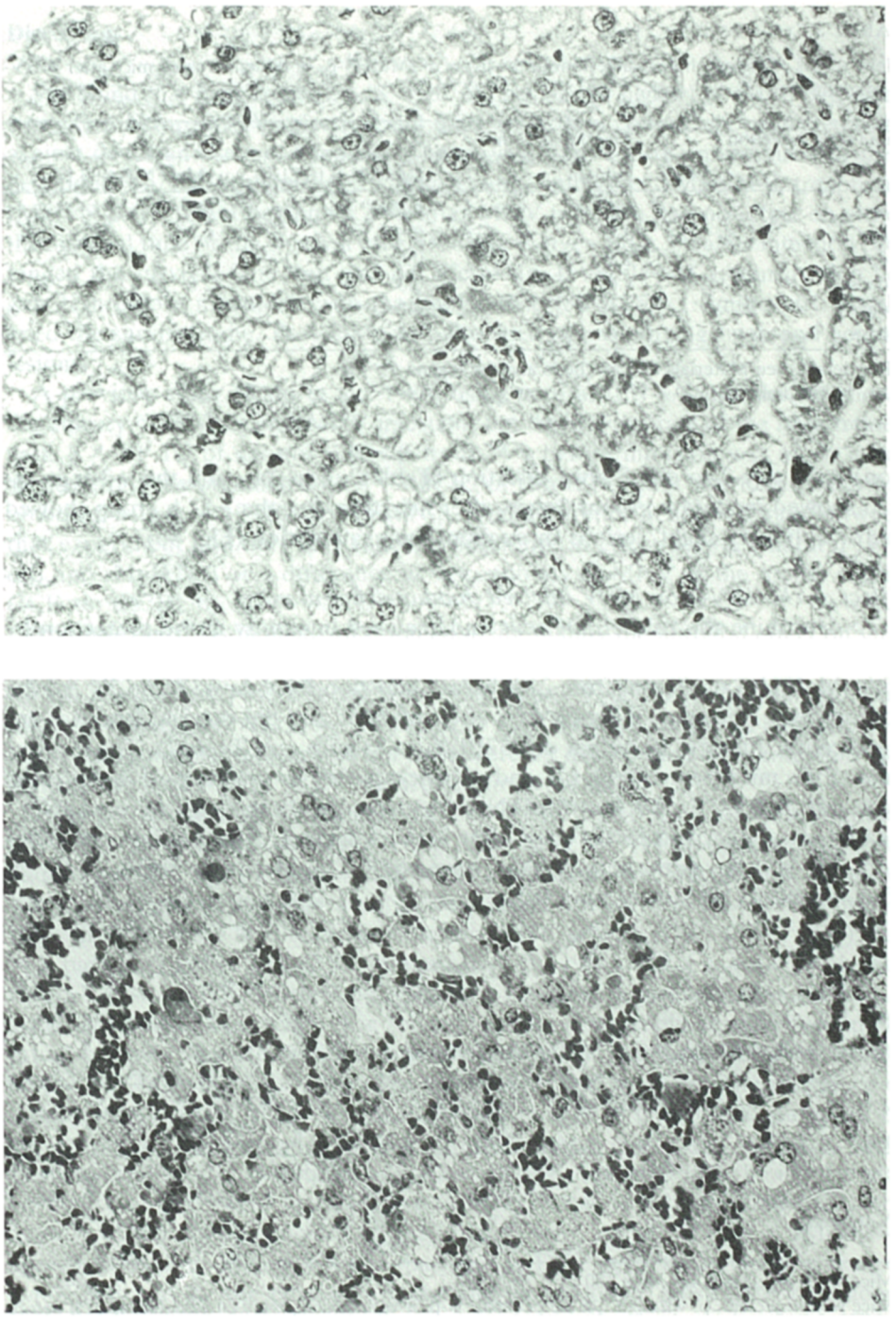

Fig. 3. Liver sections from MHV-2 infected mice (3 days after infection). (Top) Control mouse liver showing necrosis with hemorrhage (410×). (Bottom) A rMuIFN-β-treated (−21 h) mouse liver showing scattered necrosis with inflammatory cells (410×).

TABLE 2

Mean scores of histopathological lesions in the livers of rMuIFN-β- or diluent-treated mice after MHV-2 infection[a].

Time after infection	Treatment with		
	Diluent	rMuIFN-β (−21 h)	rMuIFN-β (+3 h)
8 h	0.5	N.D.	0.5
1 day	0.5	0.5	0.0
2 days	1.9	0.5	1.5
3 days	4.0	0.3	2.0
5 days	–	0.2	–
7 days	–	0.5	–

[a] For details, see Materials and Methods and the legend for Fig. 2.
N.D. = not done.

tion) from day 2 to day 3 after infection (Table 2, Fig. 3a), and the +3 h group also showed moderate lesions 3 days after infection, but the −21 h group showed no, or only very slight, lesions throughout the examination (Fig. 3b).

Effect of rMuIFN-β on MHV infected immunodeficient mice

To determine the role of NK cells and acquired immunity in the protection by rMuIFN-β, beige mice and CY-treated immunodeficient mice were treated daily with rMuIFN-β or diluent from 21 h before infection for 9 days (Table 3). All the control beige mice died within 4 days after infection and the survival time was not significantly different from that of C57BL/6(+/+) mice (see Table 1). The survival time of the mice treated with CY and diluent was shorter than that of the infected mice not given CY. All the rMuIFN-β-treated mice (both beige mice and CY-treated mice) survived for 14 days after MHV infection. Neutralizing antibodies were not detected in the sera of beige mice and CY-treated mice killed on day 28 after infection (NT: lower than 10).

TABLE 3

Effect of rMuIFN-β treatment on immunocompromised mice[a].

Mice	rMuIFN-β[b]	Mortality		Survival time (mean days ± SD)
		No.	%	
Beige	+	0/9	0	–
	–	9/9	100	3.3 ± 0.7
CY-treated[c]	+	0/10	0	–
	–	9/9	100	2.2 ± 0.4

[a] Mice were inoculated i.p. with 100 PFU of MHV-2.
[b] Mice were injected i.p. with 8×10^5 IU of rMuIFN-β or with diluent every 24 h beginning 21 h before MHV inoculation.
[c] C57BL/6 mice were injected i.p. with 200 mg of CY per kg 3 days before MHV-2 infection.

Discussion

We found that high doses (i.e., 8×10^3 IU per day or higher) of rMuIFN-β given from 21 h before MHV infection and daily for 9 days completely protected mice against death and severe hepatitis. The survival rate and the mean survival time depended on the schedule (i.e., dosage and timing) of rMuIFN-β treatment. rMuIFN-β treatment beginning 24 h after MHV infection had no effect on either the survival time or the survival rate. rMuIFN-β given from 3 h after infection prolonged the survival time, but was not lifesaving. If rMuIFN-β administration began 21 h before infection, even the lowest dose (80 IU/day) significantly lengthened the survival time. Protection was also seen against virus growth and hepatic lesions.

A previous study (Kato et al., 1986) using natural IFN-α/β has already shown the efficacy of IFNs against MHV infection, but the survival rate of the mice treated with 1×10^3 IU of natural IFN-α/β from 24 h before infection was much lower than the rate we obtained with 8×10^3 IU of rMuIFN-β and also lower than that obtained with 8×10^2 IU of rMuIFN-β. The difference in survival rate between that experiment and ours may reflect a difference in effect between natural IFN-α/β and rMuIFN-β or a difference in susceptibility of MHV of the mouse strains (ICR vs. C57BL/6).

The roles of IFNs in acute viral infection are mainly the antiviral effect on viral target cells and the immunomodulatory effects on host defense mechanisms. As for the host defense, there seems to be little, if any, role of specific immunity (antibody (Nakanaga et al., 1983; Nakanaga et al., 1986) and sensitized lymphocytes) in this experiment, because control mice were all dead 3 days after infection and rMuIFN-β was effective in CY-treated and infected mice.

IFNs are also known to augment the activity of the effectors of natural immunity (i.e., NK cells, macrophages, etc.). Schindler et al. (1982) analyzed IFN production and NK cell activation after MHV-3 infection in C57BL/6 and A/J mice, and they suggested that IFN and NK cells might not be of overwhelming importance in the defense system. Augmentation of NK activity (Djeu et al., 1979) may be a part of the effects of IFNs (Bukowski et al., 1983), but it is not critical since rMuIFN-β saved NK-deficient beige mice to the same degree as it saved C57BL/6 mice with intact NK activity. The role of peritoneal macrophages may be complicated, because they are both the target cells of MHV and the effectors of natural immunity (Taguchi et al., 1976) that can be activated by IFNs. The resistance of a given mouse strain to MHV infection could at least in part be accounted for by the genetically determined resistance of macrophages (Bang and Warwick, 1960; Taguchi et al., 1976). Virelizier and Allison (1976) reported that the in vitro susceptibility of peritoneal macrophages to MHV-3 was closely correlated with the severity of the disease induced by the virus in vivo. As the PDD_{50} of rMuIFN-β against MHV-2 in PEC was lower than that in DBT cells, the primary actions of rMuIFN-β may be the effects on macrophages and other cells of the reticuloendothelial system, but further studies are needed to clarify the role of rMuIFN-β on these cells.

High-dose rMuIFN-β therapy enabled us to save the MHV-infected susceptible mice from death. The effectiveness of rMuIFN-β against otherwise lethal MHV infection in CY-treated mice may be relevant as to the role of human IFN-β in patients with immunodeficiencies or immunosuppression who are threatened by, or suffering from, severe viral diseases.

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