Journal of Medical Virology 69:188–194 (2003)

Synergistic Antiviral Effect of a Combination of Mouse Interferon-α and Interferon-γ on Mouse Hepatitis Virus

Uichiro Fuchizaki,[1] Shuichi Kaneko,[1]* Yasunari Nakamoto,[1] Yoshihiro Sugiyama,[2] Kenichi Imagawa,[3] Mikio Kikuchi,[2] and Kenichi Kobayashi[1]

[1]*Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Ishikawa, Japan*
[2]*Third Institute of New Drug Research, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan*
[3]*Molecular Medical Science Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan*

Although interferon (IFN)-α and IFN-γ have been reported to exhibit a synergistic antiviral effect through the different signaling pathways in vitro, their therapeutic efficacy is not well defined in vivo. The current study was carried out to investigate the combined antiviral effect in a model of mouse hepatitis virus Type 2 (MHV-2) infection, in which fulminant hepatitis is developed. MHV-2 was injected intraperitoneally into 4-week-old ICR mice, IFN or the vehicle was administered intramuscularly for 5 days, and the antiviral effect was evaluated based on survival periods, liver histology, serum alanine transaminase (ALT) levels, and MHV-2 virus titers in the liver tissues. The animals in the group treated with a combination of IFN-α and IFN-γ survived for longer periods than the groups treated with IFN-α alone and IFN-γ alone (IFN-α 10^3 (IU/mouse)/-γ 10^3 vs. IFN-α 10^3, $P < 0.005$; IFN-α 10^3/-γ 10^3 vs. IFN-γ 10^3, $P < 0.001$). This is consistent with the lower levels of hepatocellular necrosis and serum ALT and the decreased titers of MHV-2 virus in the liver tissues (48 hr, $P < 0.001$; 72 hr, $P < 0.001$). These findings indicate that a combination of IFN-α and IFN-γ exhibits a synergistic antiviral effect on MHV-2 infection. The biology of MHV-2 is quite different from that of human hepatitis viruses; however, these results suggest the beneficial combined therapy of IFN-α and IFN-γ for the treatment of human viral hepatitis. ***J. Med. Virol. 69:188–194, 2003.*** © 2003 Wiley-Liss, Inc.

KEY WORDS: mouse hepatitis virus; interferon-α; interferon-γ; antiviral effect; synergy

INTRODUCTION

Interferon (IFN)-α is used widely for the treatment of chronic hepatitis C. The therapeutic effect of IFN-α is determined by the virus titer to some extent, but various other factors are considered to be involved [Martinot-Peignoux et al., 1995; Enomoto et al., 1996; Mizokami et al., 1996]. To improve the therapeutic effect on patients with IFN-α-resistant chronic hepatitis C, types of IFN-α [Heathcote et al., 1998; Zeuzem et al., 2000], administration period [Kasahara et al., 1995], and administration method [Marcellin et al., 1995; Fujiwara et al., 1998] have been investigated, and new therapeutic methods such as combination therapy with IFN and ribavirin [Davis et al., 1998; McHutchison et al., 1998], and viral gene-targeting therapy [Macejak et al., 2000] have been attempted. Sufficient efficacy has not been attained, however, and a new therapeutic method that improves further the efficacy of IFN therapy is needed.

IFN is known to activate 30 or more IFN-stimulated genes and the antiviral effect is exhibited by the proteins induced by IFN, such as 2′-5′ oligoadenylate synthetase (2′-5′ OAS), double-strand RNA-dependent protein kinase (PKR), and Mx proteins [Samuel, 1991; Der et al., 1998]. IFN is classified into Type I IFN (IFN-α, -β, -ω) and Type II IFN (IFN-γ), and IFN-α consists of many subtypes and molecular forms. Type I and Type II IFN signal via different receptors: IFNAR1 and IFNAR2 for Type I and IFNGR1 and IFNGR2 for Type II [Allen et al., 1996]. It has been reported that although the signal transduction pathways differ between IFN-α and IFN-γ [Darnell et al., 1994], synergistic gene expression is induced by a combination of IFN-α and IFN-γ [Levy et al., 1990; Thomas et al., 1992; Matsumoto et al., 1999; Mizukoshi et al., 1999].

*Correspondence to: Dr. Shuichi Kaneko, Department of Gastroenterology, Kanazawa University Graduate School of Medicine, 13-1 Takara-Machi, Kanazawa, 920-8641, Japan.
E-mail: skaneko@medf.m.kanazawa-u.ac.jp

Accepted 27 June 2002
DOI 10.1002/jmv.10286
Published online in Wiley InterScience (www.interscience.wiley.com)

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Although combination therapy with IFN-α and IFN-γ has been studied clinically [Katayama et al., 2001; Kumashiro et al., 2002], the effect and action mechanism have not been investigated fully. The current study was undertaken to investigate whether concurrent administration of IFN-α and IFN-γ increases the antiviral effect in a model of mouse hepatitis virus Type 2 (MHV-2) infection.

MATERIALS AND METHODS

Propagation and Quantitation of MHV-2

Mouse hepatitis virus (MHV) is a 31-kb positive single-stranded RNA virus belonging to Coronaviridae [Lee et al., 1991; Compton et al., 1993]. In this experiment, MHV-2 (provided by Professor S. Kyuwa, Department of Animal Pathology, Institute of Medical Science, University of Tokyo) was used, which is known to cause fulminant hepatitis for inbred ICR mouse strain [Jones and Cohen, 1962].

MHV-2 was propagated and quantitated according to the method reported by Hirano et al. [1976, 1981], using murine delayed brain tumor (DBT) cells (provided by Professor S. Kyuwa). DBT cells were cultured in Eagle's minimum essential medium (MEM; GIBCO BRL, Rockville, MD) containing 10% each of FCS (GIBCO BRL) and tryptase phosphate broth (TPB; GIBCO BRL) at 37°C under 5% CO_2.

During the propagation of MHV-2, the culture medium was discarded when DBT cells formed a monolayer in a 25-cm^2 culture dish. After 0.2 ml of MHV-2 stock solution was adsorbed to DBT cells at 37°C for 1 hr, 5 ml of culture medium was added, and the cells were cultured for 15 hr. The obtained culture fluid was frozen at −80°C as MHV-2 stock solution until immediately before the experiment.

For quantitation of MHV-2, a monolayer of DBT cells in a 35-mm Petri dish was infected with 0.2 ml of an MHV-2 sample for 45 min and then MEM (first overlay medium) containing 10% TPB, 5% FCS, and 1% Noble agar (Difco Laboratories, Detroit, MI) was added. After the cells were cultured for another 2 days, MEM (secondary overlay medium) containing 0.01% neutral red was added, and the number of plaques was measured after 8 hr.

MHV-2-Induced Hepatitis Model

A mouse hepatitis model was prepared by single intraperitoneal inoculation of 0.2 ml with MHV-2 suspension adjusted to contain 10^3 plaque-forming units (PFU)/0.2 ml of MHV-2 with culture medium to 4-week-old male ICR mice (Charles River Japan, Yokohama, Japan). Animal care and procedures were carried out using criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23, revised 1985).

All groups consisted of 20 animals. In the IFN treatment groups, IFN was administered intramuscularly (i.m.) to the femoral muscle daily from 1 day before MHV-2 inoculation to 3 days after inoculation. IFN was dissolved in and adjusted with 0.1% mouse albumin-supplemented physiological saline. For IFN, recombinant mouse interferon (rmIFN) was provided by Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The IFN dosages in the group treated with a single type of IFN were IFN-α (10^3, 10^4, and 10^5 IU/50 μl/mouse), IFN-γ (10^3, 10^4 IU/50 μl/mouse), and in the combination treatment groups were: IFN-α 10^3/-γ 10^3 and IFN-α 10^4/-γ 10^4 IU/50 μl/mouse. The control group received administration of the same volume of vehicle (0.1% mouse albumin-supplemented physiological saline).

Survival Period

The survival period (days) after MHV-2 inoculation was investigated in each group. For simple comparison of the survival period, Kaplan-Meier survival curves were used. The survival period was analyzed by the survival period test program using SAS system (SAS Institute Japan R.6.12). To determine the synergism, the survival period tested by the survival period test program by two-way layout ANOVA using SAS system was compared among the groups and among the total IFN units administered. When the survival curve was prolonged significantly in the combination treatment groups in both comparisons, the effect was judged synergistic.

Liver Histology

In the control group, groups treated with a single type of IFN (IFN-α 10^3, IFN-γ 10^3), and combination treatment group (IFN-α 10^3/-γ 10^3) (n = 20 in all groups), three animals were sacrificed daily until 3 days after the MHV inoculation in each group. The liver tissues were stained with hematoxylin and eosin at each time point and examined under a light microscope.

Serum ALT Level and MHV-2 Virus Titer in Liver Tissue

In the control group, the groups treated with a single type of IFN (IFN-α 10^3, IFN-γ 10^3), and the combination treatment group (IFN-α 10^3/-γ 10^3) (n = 30 in all groups), animals were killed 2 and 3 days after MHV-2 inoculation in each group, and the serum alanine transaminase (ALT) level and MHV-2 virus titer in the liver tissue were investigated. The serum ALT level was measured using Transaminase CII-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). To measure the MHV-2 virus titer in the liver tissue, 0.2 g of the wet liver tissue was homogenized in 0.8 ml of culture medium, and MHV-2 was quantified by plaque assay.

To avoid variation among assays, mice supplied at the same time were divided into the groups, and IFN administration and MHV-2 inoculation were carried out on the same days in all groups.

Statistical Analysis

Values were expressed as the mean ± SD. Comparison between two groups was analyzed by Wilcoxon's rank-sum test. The synergistic effect on the MHV-2 virus titer was analyzed by two-way ANOVA using SAS system. A P-value of <0.05 was considered significant.

RESULTS

Survival Period of MHV-2 Infected Mice

In the control group, all animals died within 4 days after MHV-2 inoculation (Fig. 1A). In the groups treated with IFN-α alone (10^3, 10^4, and 10^5 IU/mouse), the survival period was prolonged significantly compared to the control group (IFN-α 10^3, $P < 0.005$; IFN-α 10^4, $P < 0.001$; IFN-α 10^5, $P < 0.001$) (Fig. 1A). Similarly, in the groups treated with IFN-γ alone (10^3 and 10^4 IU/mouse), the survival period was significantly prolonged (IFN-γ 10^3, $P < 0.05$; IFN-γ 10^4, $P < 0.001$) (Fig. 1B).

Interestingly, the animals treated with a combination of IFN-α 10^3 and IFN-γ 10^3 (IU/mouse) survived for longer periods than animals treated with IFN-α 10^3 alone and IFN-γ 10^3 alone (IFN-α 10^3/-γ 10^3 vs. IFN-α 10^3, $P < 0.005$; IFN-α 10^3/-γ 10^3 vs. IFN-γ 10^3, $P < 0.001$), and the effect was synergistic ($P < 0.001$) (Fig. 2A). When the doses of IFN-α and IFN-γ were increased, the survival period was also prolonged in the IFN-α 10^4/-γ 10^4 combination group compared to the group treated with either of IFN (Fig. 2B).

Liver Histology of MHV-2 Infected Mice

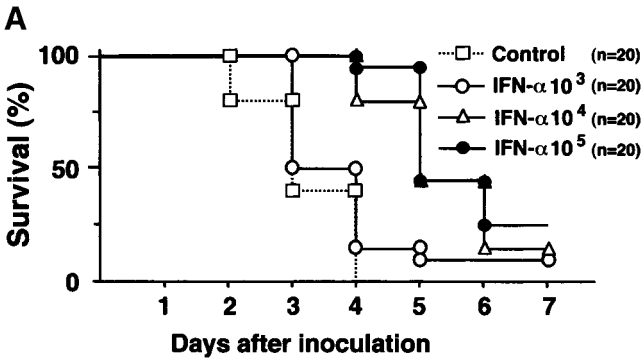
In the control group, submassive or massive necrosis was observed in the liver tissue 2 days after MHV-2 inoculation, and hepatocellular necrosis progressed with replication of MHV-2 in the liver (Fig. 3). In the group treated with IFN-γ 10^3 alone, the severity was almost at the same level as those in the control group. In contrast, in the group treated with IFN-α 10^3 alone, spotty necrosis was observed in one of three animals 2 days after MHV-2 inoculation, but no hepatocellular necrosis was observed in the other two animals. In the IFN-α 10^3/-γ 10^3 combination group, hepatocellular necrosis was not observed until 2 days after MHV-2 inoculation, and spotty necrosis was observed in two of three animals 3 days after inoculation; however, hepatocellular necrosis was not observed in the remaining one animal. In the groups treated with IFN-α 10^3 alone and with IFN-α 10^3/-γ 10^3 combination, hepatocellular necrosis was obviously mild compared to the control group (Fig. 3, Table I).

Serum ALT Level of MHV-2 Infected Mice

The serum ALT levels 2 and 3 days after MHV-2 inoculation in the control group (Day 2, $n = 5$; Day 3, $n = 4$) and the groups treated with IFN-α 10^3 alone (Day 2, $n = 15$; Day 3, $n = 13$), IFN-γ 10^3 alone (Day 2, $n = 14$; Day 3, $n = 8$), and IFN-α 10^3/-γ 10^3 combination (Day 2, $n = 14$; Day 3, $n = 15$) are shown in Figure 4. The serum ALT levels 2 days after MHV-2 inoculation were significantly lower in the group treated with IFN-α 10^3/-γ 10^3 combination compared to the groups treated with IFN-α 10^3 alone and IFN-γ 10^3 alone (IFN-α 10^3/-γ 10^3 vs. IFN-α 10^3, $P < 0.005$; IFN-α 10^3/-γ 10^3 vs. IFN-γ 10^3, $P < 0.005$). The serum ALT levels 3 days after MHV-2 inoculation were significantly lower in the group treated with IFN-α 10^3/-γ 10^3 combination compared to the group treated with IFN-α 10^3 alone (IFN-α 10^3/-γ 10^3 vs. IFN-α 10^3, $P < 0.05$; IFN-α 10^3/-γ 10^3 vs. IFN-γ 10^3, $P = 0.068$).

MHV-2 Virus Titers in Liver Tissues

The MHV-2 titers in the liver tissue 2 and 3 days after MHV-2 inoculation in the control group (Day 2, $n = 5$; Day 3, $n = 4$) and the groups treated with IFN-α 10^3 alone (Day 2, $n = 15$; Day 3, $n = 13$), IFN-γ 10^3 alone (Day 2, $n = 14$; Day 3, $n = 8$), and IFN-α 10^3/-γ 10^3



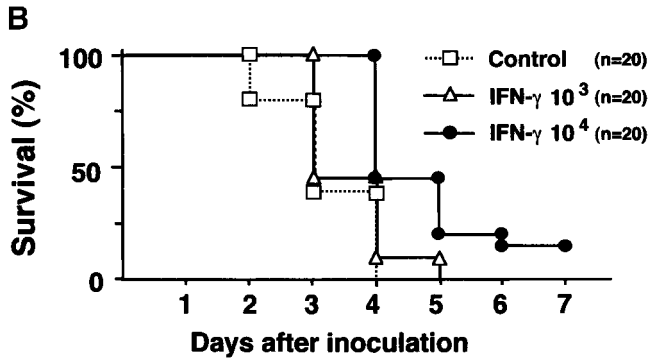
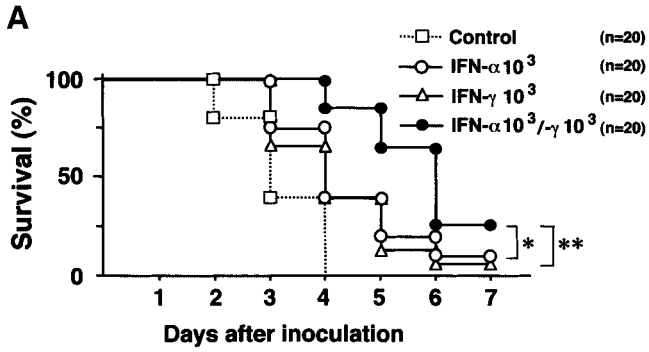


Fig. 1. **A:** Protective effect of IFN-α alone on the survival of mice infected with MHV-2. Four-week-old male ICR mice were infected i.p. with 10^3 PFU MHV-2/mouse. IFN-α ($n = 20$) was injected i.m. at doses of 10^3 (○), 10^4 (△), and 10^5 IU/50 μl/mouse (●). Control mice ($n = 20$) were injected i.m. with the same volume of vehicle (0.1% mouse albumin-supplemented physiological saline; □). A dose of 10^3 PFU MHV-2 killed 100% of mice within 4 days. In IFN-α 10^3 ($P < 0.05$), IFN-α 10^4 ($P < 0.001$), and IFN-α 10^5 ($P < 0.001$), survival was prolonged significantly compared to control. **B:** Protective effect of IFN-γ alone on survival of mice infected with MHV-2. IFN-γ ($n = 20$) was injected i.m. at doses of 10^3 (△), 10^4 IU/50 μl/mouse (●). Control mice (n = 20) were injected i.m. with the same volume of vehicle (□). In IFN-γ 10^3 ($P < 0.05$), IFN-γ 10^4 ($P < 0.001$), survival was prolonged significantly compared to control.



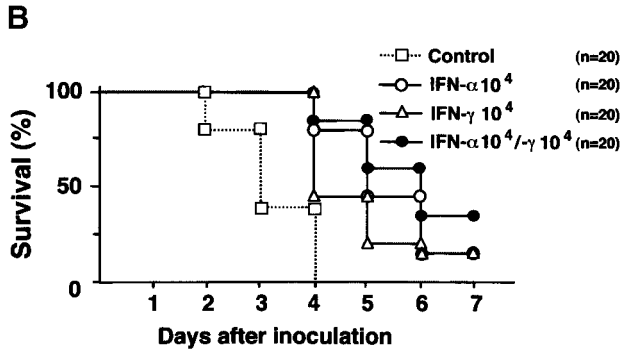


Fig. 2. **A:** Protective effect of a combination treatment with IFN-α and IFN-γ. IFN-α alone ($n = 20$) was injected i.m. at doses of 10^3 (○). IFN-γ alone ($n = 20$) was injected i.m. at doses of 10^3 IU/50 μl/mouse (△). IFN-α and IFN-γ ($n = 20$) combination was injected i.m. at doses of IFN-α 10^3 and IFN-γ 10^3 IU/50 μl/mouse (●). Control mice ($n = 20$) were injected i.m. with the same volume of the vehicle (□). In IFN-α and IFN-γ combination, survival was prolonged significantly compared to IFN-α alone ($P < 0.005$) and IFN-γ alone ($P < 0.001$). *$P < 0.005$; **$P < 0.001$. **B:** IFN-α alone ($n = 20$) was injected i.m. at doses of 10^4 IU/50 μl/mouse (○). IFN-γ alone ($n = 20$) was injected i.m. at doses of 10^4 IU/50 μl/mouse (△). IFN-α and IFN-γ ($n = 20$) combination was injected i.m. at doses of IFN-α 10^4 and IFN-γ 10^4 IU/50 μl/mouse (●). Control mice ($n = 20$) were injected i.m. with the same volume of vehicle (□). In IFN-α and IFN-γ combination, survival was prolonged compared to IFN-α alone ($P = 0.2287$) and IFN-γ alone ($P < 0.05$).

combination (Day 2, $n = 14$; Day 3, $n = 15$) are shown in Figure 5. In the control group, the MHV-2 titers in the liver tissue 2 and 3 days after MHV-2 inoculation were 7.67 ± 1.45 and 9.07 ± 0.30 ($\log_{10}$ PFU/g · wet tissue), respectively. In the group treated with IFN-α 10^3 alone, the MHV-2 titers in the liver tissue 2 and 3 days after MHV-2 inoculation were 6.16 ± 1.31 and 7.32 ± 1.26 ($\log_{10}$ PFU/g · wet tissue), respectively. In the group

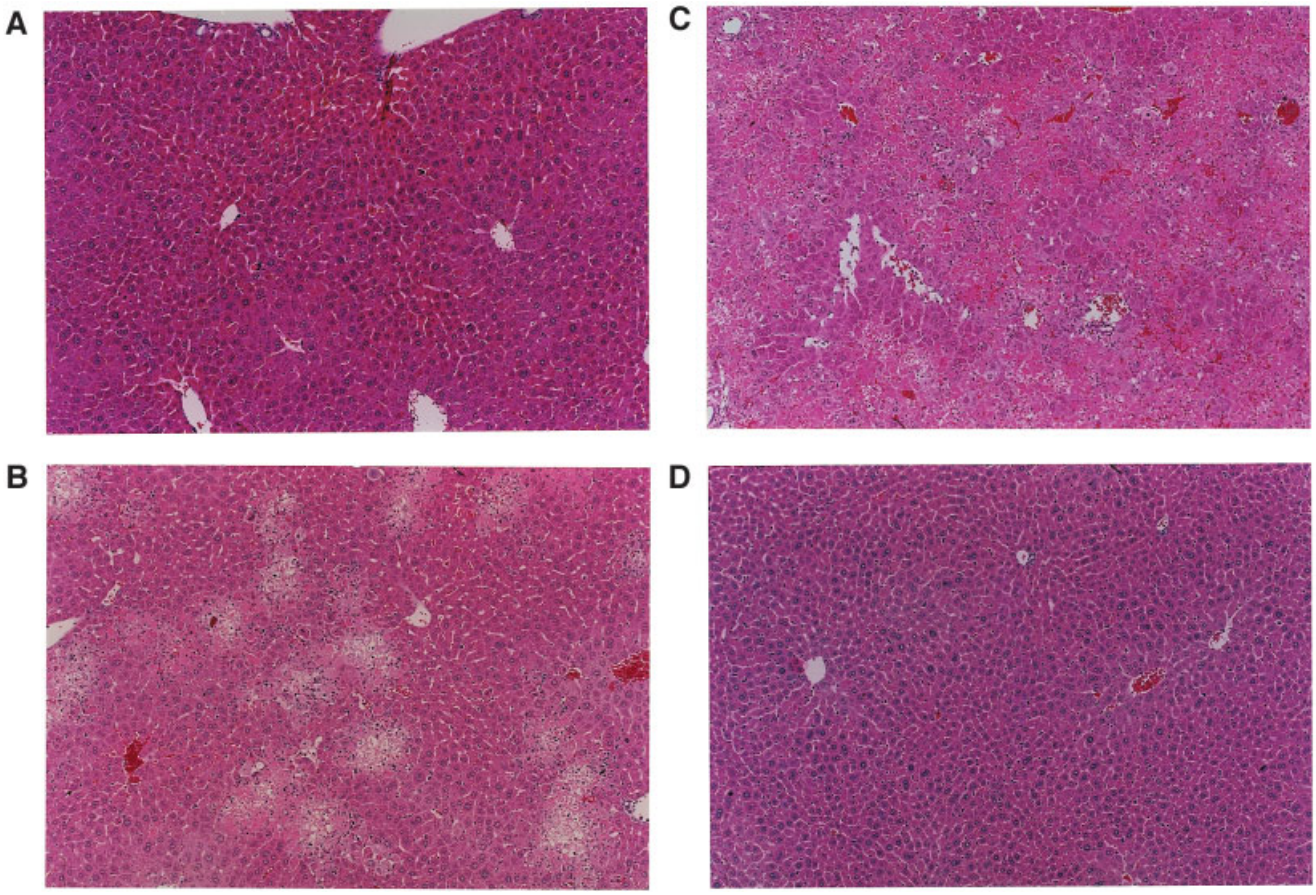


Fig. 3. Histological changes in MHV-2 infected mice. **A:** Representative liver of control mouse at 1 day after MHV-2 infection showing spotty necrosis. **B:** Representative liver of control mouse at 2 days after MHV-2 infection showing focal necrosis of the liver infected MHV-2. **C:** Representative liver of control mouse at 3 days after MHV-2 infection showing massive necrosis. **D:** Representative liver of IFN-α 10^3 and IFN-γ 10^3-treated mouse at 3 days after MHV-2 infection showing no focal necrosis (Hematoxylin-eosin staining; original magnification ×200.)

TABLE I. Degree of Hepatocellular Necrosis*

Treatment	Day 1 (n)	Day 2 (n)	Day 3 (n)
Control	(−): 1	(2+): 2	(3+): 1
	(+): 2	(3+): 1	(4+): 2
IFN-γ 10^3	(−): 2	(2+): 2	(3+): 2
	(+): 1	(3+): 1	(4+): 1
IFN-α 10^3	(−): 3	(−): 2	(2+): 2
		(+): 1	(3+): 1
IFN-α 10^3/γ 10^3	(−): 3	(−): 3	(−): 1
			(+): 2

*(+), Spotty necrosis; (2+), focal necrosis; (3+), submassive necrosis; (4+), massive necrosis. Mice were treated with IFN-α 10^3 IU/50 ml per mouse ($n = 20$) and IFN-γ 10^3 IU/50 ml per mouse ($n = 20$) alone or in combination ($n = 20$). Control mice were injected i.m. with the same colume of vehicle. In IFN-α alone and IFN-α/-γ combination, the degree of hepatocellular necrosis was milder than in the control.

treated with IFN-γ 10^3 alone, the MHV-2 titers in the liver tissue 2 and 3 days after MHV-2 inoculation were 6.38 ± 0.79 and 8.02 ± 1.59 ($\log_{10}$ PFU/g · wet tissue), respectively. In the group treated with IFN-α 10^3/-γ 10^3 combination, the MHV-2 titers in the liver tissue 2 and 3 days after MHV-2 inoculation were 5.48 ± 0.90 and 6.21 ± 0.54 ($\log_{10}$ PFU/g · wet tissue), respectively. A comparison among the control group and the groups treated with IFN-α 10^3 alone, IFN-γ 10^3 alone, and IFN-α 10^3/-γ 10^3 combination shows that the MHV-2 virus titer in the liver tissue decreased significantly in the group treated with IFN-α 10^3/-γ 10^3 combination at both 2 and 3 days after MHV-2 inoculation, and the effect was synergistic (Day 2, $P < 0.001$; Day 3, $P < 0.001$; Fig. 5).

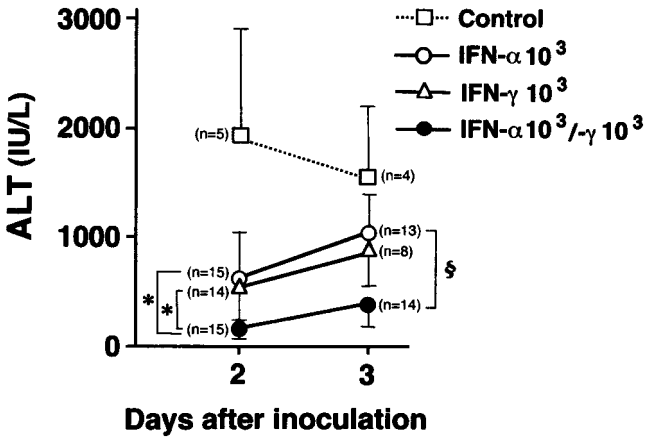


Fig. 4. Comparison of changes in serum ALT levels over the time course. Mice were treated with IFN-α 10^3 (○; Day 2, $n = 15$; Day 3, $n = 13$) and IFN-γ 10^3 (△; 48 hours, $n = 14$; Day 3, $n = 8$), alone or in combination (●; Day 2, $n = 14$; Day 3, $n = 15$). Control mice were injected i.m. with the same volume of vehicle (□; Day 2, $n = 5$; Day 3, $n = 4$). Data are expressed as the mean ± SD of serum ALT. At 2 days after MHV-2 infection, the serum ALT levels of IFN-α 10^3 and IFN-γ 10^3-treated mice were significantly lower than that of both IFN-α 10^3-treated mice ($P < 0.005$) and IFN-γ 10^3-treated mice ($P < 0.005$). At 3 days after MHV-2 inoculation, the serum ALT level decreased significantly compared to the group treated with IFN-α 10^3 alone (IFN-α 10^3/-γ 10^3 vs. IFN-α 10^3, $P < 0.05$; IFN-α 10^3/-γ 10^3 vs. IFN-γ 10^3, $P = 0.068$). § $P < 0.05$; *$P < 0.005$.

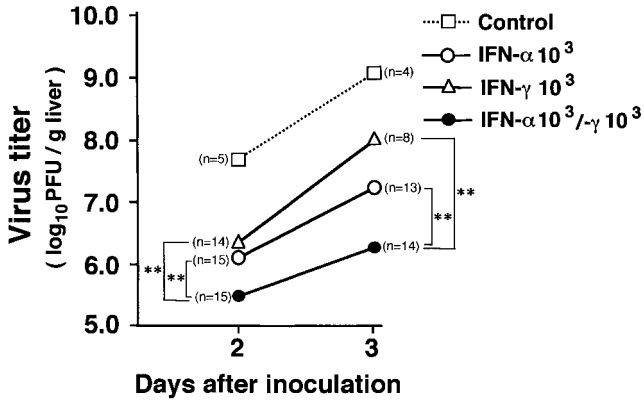


Fig. 5. Antiviral effect of treatment with IFN on MHV-2 replication. Mice were treated with IFN-α 10^3 (○; Day 2, $n = 15$; Day 3, $n = 13$) and IFN-γ 10^3 (△; Day 2, $n = 14$; Day 3, $n = 8$), alone or in combination (●; Day 2, $n = 14$; Day 3, $n = 15$). Control mice were injected i.m. with the same volume of vehicle (□; Day 2, $n = 5$; Day 3, $n = 4$). The MHV-2 virus titer in the liver tissue decreased significantly in the group treated with IFN-α 10^3/-γ 10^3 combination at each time point compared to the groups treated with IFN-α 10^3 alone and IFN-γ 10^3 alone, and the effect was synergistic (Day 2, $P < 0.001$; Day 3, $P < 0.001$, two-way ANOVA using SAS system). **$P < 0.001$. Liver homogenates were prepared, and virus titers were determined by plaque assay.

DISCUSSION

A mouse hepatitis virus Type 2 (MHV-2)-induced hepatitis model was used to determine the potentiation of antiviral effect by a combination of IFN-α and IFN-γ. It has been reported that exogenous mIFN-α/β administration prolongs survival in a mouse MHV-2-induced hepatitis model [Kato et al., 1986]. It has been reported also that the administration of rIFN-α/β 24 hr before or simultaneously with MHV inoculation is effective in a chronic hepatitis model induced by MHV-2cc, which is an attenuated virus [Uetsuka et al., 1996]. IFN-γ receptor deficient mice have been shown to be infected easily with MHV [Schijns et al., 1996]. Kyuwa et al. [1998a,b] showed that the administration of rIFN-γ prolonged the survival of mouse-hepatitis virus strain JHM-infected IFN-γ receptor deficient C57BL/6 mice, noting the importance of IFN-γ in the elimination of the virus. Zhang et al. [1997] showed in vitro that the antiviral effects of IFN-γ on MHV occurred when IFN-γ was administered 24 hr before MHV inoculation. Based on this finding, IFN administration was initiated 1 day before MHV-2 inoculation in this study. The survival period was prolonged in a dose-dependent manner in the groups treated with IFN-α alone, and the survival curve was prolonged significantly compared to the control group. In the groups treated with IFN-γ alone, a significant prolongation of the survival curve was observed compared to the groups treated with IFN-α alone, although the statistical significance was marginal, confirming the prolongation of life by IFN. Interestingly, the animals in the group treated with a combination of IFN-α and IFN-γ survived for longer periods than the groups treated with IFN-α alone and IFN-γ alone.

Histological examination of the liver tissues, biochemical examination, and quantitation of MHV-2 in the liver tissues were carried out in the groups treated with IFN-α 10^3/-γ 10^3 combination, IFN-α 10^3 alone, and IFN-γ 10^3 alone. On the histological examination, the degrees of hepatocellular necrosis were mild in the groups treated with IFN-α alone and IFN-α/-γ combination compared to the control group, indicating that the progression of hepatocellular necrosis was inhibited by IFN administration. This finding was consistent with significant decreases of serum ALT levels 2 days after MHV-2 inoculation. The MHV titers were considered to be the major cause of hepatocellular necrosis. In the MHV-2 hepatitis model, the amount of virus is considered to determine the degree of hepatocellular necrosis and survival rate. We investigated whether the MHV-2 virus titer in liver tissue was decreased by the administration of IFN, and it was found that the MHV-2 virus titer decreased significantly at each time point in the group treated with IFN-α/-γ combination. Therefore, it was shown that an exogenous administration of IFN-α/-γ combination synergistically inhibited viral titer in the liver, which may not have inhibited the progression of hepatocellular necrosis only, but also prolonged survival in a synergistic manner. Liver damage, however, measured as the serum ALT levels, was not reduced synergistically. One reason for this discrepancy is because low titer of MHV-2 can still induce mild hepatocellular necrosis, despite the fact that viral titer in the liver was lowered ten-fold with respect to IFN-α or IFN-γ alone.

The interaction mechanism between IFN-α and IFN-γ, activation of IFN-stimulated gene factor 3 (ISGF3) by IFN-γ has been shown [Matsumoto et al., 1999]. Levy et al. [1990] reported treatment of HeLa cells with IFN-γ; IFN-α then induced increased synthesis of latent ISGF3, which was subsequently activated in response to IFN-α to form levels ~10-fold higher than levels detected in cells treated with IFN-α alone, showing a synergistic effect. It was shown that IFN-γ induced expression of IFNAR-1 and IFNAR-2 mRNA, and treatment with IFN-γ followed by the administration of IFN-α, significantly increased the intracellular 2′-5′ OAS activity compared to that after treatment with IFN-α alone [Mizukoshi et al., 1999]. Takaoka et al. [2000] found a new signal cross talk system between IFN-α and IFN-γ at the receptor level in caveola membrane domains. In IFNAR1-deficient mouse embryonic fibroblasts, activation of Stat1, ISGF3 complex and antiviral activity were decreased, and IFNAR1 was phosphorylated; the amounts of Stat1 and Stat2, which recruit to IFNAR1, were increased by stimulation with IFN-γ. They showed that Stat2 was activated via IFNGR2-associated IFNAR1, noting that signal transduction of IFN-γ may be made more potent and efficient using the system via the IFN-α/β receptors, and that the synergistic antiviral effect of IFN-α and IFN-γ was caused by this system. It is not clear whether the mechanism described above was reflected directly in this study but the synergistic gene expression induced by a combination of IFN-α and IFN-γ may have potentiated the antiviral effect, suggesting that investigation of the expression levels of ISGF3 and 2′-5′ OAS is necessary.

Regarding the immunological background, it has been reported that in MHV-3-induced hepatitis models, A/J mouse, in which Type 1 T-helper (Th1) cytokines are activated, is resistant to MHV-3, whereas BALB/c mice, in which Th2 cytokines are activated, are easily infected with MHV-3 [Liu et al., 1998]. In this study, the action of Th1 cytokine induced by IFN-γ may have been effective for virus elimination.

In addition to the in vitro synergistic gene expression induced by concurrent administration of IFN-α and IFN-γ, the present study showed the synergistic antiviral effect of combination therapy with IFN-α and IFN-γ against coronavirus in vivo. There are several publications that argue against the use of IFN-α and IFN-γ combination therapy in human viral hepatitis [Krampera et al., 2000; Bergamini et al., 2001; Kumashiro et al., 2002]. Further investigation is necessary, however, because this study suggests that combination therapy with IFN-α and IFN-γ may become a therapeutic method for treatment of viral hepatitis in man, a method in which the synergistic antiviral effect may improve the efficacy of IFN therapy.

ACKNOWLEDGMENTS

The authors thank Professor S. Kyuwa, Department of Animal Pathology, Institute of Medical Science, University of Tokyo, for providing MHV-2 and DBT cells. We also thank Dr. M. Fujii, Dr. K. Yamamoto, and Dr. H. Yamauchi at Hayashibara Biochemical Laboratories, Inc. for providing mouse interferon, and the staff members of the second laboratory of the Department of Gastroenterology, Kanazawa University Graduate School of Medicine, for their cooperation.

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