Antiviral Research, 7 (1987) 289–301
Elsevier

AVR 00242

Protection against herpes simplex virus infection in mice by recombinant murine interferon-β in combination with antibody

Yuji Kumano, Masahiro Yamamoto and Ryoichi Mori

Department of Virology, School of Medicine, Kyushu University, Fukuoka, Japan

(Received 29 December 1986; accepted 28 February 1987)

Summary

A recombinant murine interferon -β (rMuIFN-β) was used to suppress the development of skin lesions and death of mice after challenge with herpes simplex virus (HSV) type 1 (HSV-1).

Depilated female BALB/c mice were inoculated intradermally with HSV-1, Hayashida strain, and were administered various concentrations of interferon (IFN) intraperitoneally 3 h later. The treatment with IFN was given once a day for 10 successive days. Under the conditions in which almost all control mice died after development of severe zosteriform skin lesions, the mortality of mice treated with IFN (8×10^5 or 8×10^4 U/mouse) was less than 50% (9/20 and 4/10, respectively), though all mice treated with a lower dose of IFN (8×10^3 U/mouse) died. Titration revealed that there was no significant suppression of virus growth by IFN in the skin or dorsal root ganglia, but it was significantly suppressed in the brain.

The protective effect of IFN was enhanced when it was used in combination with human anti-HSV antibody having a neutralizing titer (NT) of 1:16. All mice treated with IFN (8×10^5 U/mouse) and antibody (NT, 1:16) survived, and only 40% of them developed slight zosteriform skin lesions. The effect of the combination was observed even when both IFN and antibody were diluted 1:10.

The protective effect of IFN was also observed when athymic nude mice were used as the host. In this system, though the IFN-treated nude mice survived significantly longer than the controls, they finally died. In antibody- or acyclovir

Correspondence to: Prof. R. Mori, Department of Virology, School of Medicine, Kyushu University, Fukuoka 812, Japan.

(ACV)-treated nude mice, there was also a prolongation of survival time as compared with control mice. The effect of antibody was enhanced by the addition of IFN, but IFN did not potentiate the effect of ACV.

Herpes simplex virus; Recombinant interferon; Interferon-β; Antibody; Acyclovir

Introduction

The effects of exogenous interferon (IFN) on infection with herpes simplex virus (HSV) have been investigated in animal models and patients [4,5,19,22,26]. However, in general, good antiherpetic effects have not been obtained. Until recently, most studies with interferons (IFNs) were conducted with relatively impure preparations generated by infecting leukocytes or fibroblasts with viruses, or stimulating lymphocytes with mitogens. Therefore the antiviral effect of exogenous IFN has not yet been sufficiently evaluated. Because of recent advances in genetic engineering, recombinant IFN has become available in large quantities, and it should be worthwhile to study the antiviral effect of the recombinant IFN in animal models. There are several reports of the use of human recombinant IFN in HSV-infected mice [8,17]. However, as IFN is species specific, homologous murine IFN should be used to determine the antiviral effect in mice. Studies using homologous murine recombinant IFN in mice are rare [9], and the antiviral effect of recombinant IFN-β has also not yet been studied.

Recently, the effects of combinations of two or more antiviral agents against HSV infection have been investigated in vitro and in vivo [1,3,9,14,18,20]. It has been shown that the use of IFN in combination with acyclovir (ACV) enhances the antiviral effect in HSV-infected mice. We have also shown that the combined use of ACV and antibody is highly effective against HSV infection in nude mice [27].

In the present study, we evaluated the antiviral effects of recombinant murine IFN-β (rMuIFN-β) in depilated mice or athymic nude mice infected intradermally with HSV. Intradermal infection of depilated or nude mice was used to assess the efficacy of antiherpes drugs, because this system has the advantage that skin lesions are readily visible and, to a certain extent, can be assessed quantitatively [21,24]. Furthermore, combinations of IFN with other antiviral agents having different mechanisms of action, i.e., antibody or ACV, were investigated.

Materials and Methods

Virus

HSV type 1 (HSV-1), strain Hayashida, an isolate from a vesicular skin lesion of herpes labialis, was passaged 7 times in Vero cells and used for inoculation. Vero cells grown in Eagle's minimal essential medium (MEM) supplemented with 5% calf serum were used for virus propagation.

Mice

Euthymic female BALB/c mice were obtained from the Kyudo Co., Ltd., Kumamoto, Japan and were used as immunocompetent mice. Female athymic nude mice with BALB/c genetic background were purchased from the Central Laboratory of Experimental Animals, Co., Ltd., Osaka, Japan. They were infected at 5–7 wk of age.

Inoculation of mice

The right midflank of each BALB/c mouse was clipped and depilated with a chemical depilatory, Hair Remover (Shiseido, Co., Ltd., Tokyo, Japan). Ten scarifications were made with a 26 gauge hypodermic needle at the midflank of the depilated mice or nude mice and 20 μl containing 1×10^6 plaque-forming units (pfu) of HSV-1 was dropped on the scarified area. Development of the skin lesions was observed and scored every day. Scores of skin lesions are as follows: O, no lesion; 2, local lesion (vesicle or erosion);6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and death.

Interferon

rMuIFN-β was provided by Basic Research Laboratories, Toray Industries Inc., Tokyo, Japan. The activity of rMuIFN-β was assayed by the cytopathic effect inhibition method using vesicular stomatitis virus and mouse L cells, and the titer was expressed in international reference units (U) based on the international standard MuIFN-α/β (G-002-904, 511, National Institutes of Health, Bethesda, MD, U.S.A.). The activity of the preparations of rMuIFN-β was 1.0×10^7 U/ml.

Antibody

Heat-inactivated human serum was used as antibody and had a neutralizing titer (NT) of 1:16 against HSV-1. The neutralizing activity of serum was measured by the microplate method described previously [21], where the neutralizing antibody titer was expressed as the highest dilution which showed 80% plaque reduction.

Acyclovir

ACV was brought in suspension in phosphate-buffered saline, pH 7.2, at a concentration of 5 mg/ml.

Cocultivation of dorsal root ganglia with Vero cells

Mice that survived 50 days after inoculation were sacrificed. The dorsal root ganglia (thoracolumbar area) were removed and were put onto monolayers of Vero cells. The cultures were examined daily for 4 wk for the appearance of viral cytopathic effect.

Virus titrations

At various intervals after virus inoculation, three mice from each group were sacrificed and viral titers in their tissues were determined as described previously [27]. The mice were exsanguinated by cutting the femoral vessels before the tissues were removed. Tissue samples included the skin (the minimum area encompassing the inoculated site and the whole lesion), dorsal root ganglia (thoracolumbar area), and brain. Homogenates of the tissues were inoculated onto Vero cell monolayers in 24-well tissue culture plates and overlaid with MEM containing 2% calf serum and 2% methylcellulose. After incubation for 3 days, plaques were counted. The titers of virus recovered from the various homogenates were expressed as pfu per tissue.

Statistical analyses

To compare the mortality of control and IFN-treated mice, the data were evaluated using the Fisher exact test. Statistical evaluations of differences between the mean survival times and differences in virus titers between control and IFN-treated mice were evaluated using the Student's *t*-test. A p value of 0.05 or less was considered as significant.

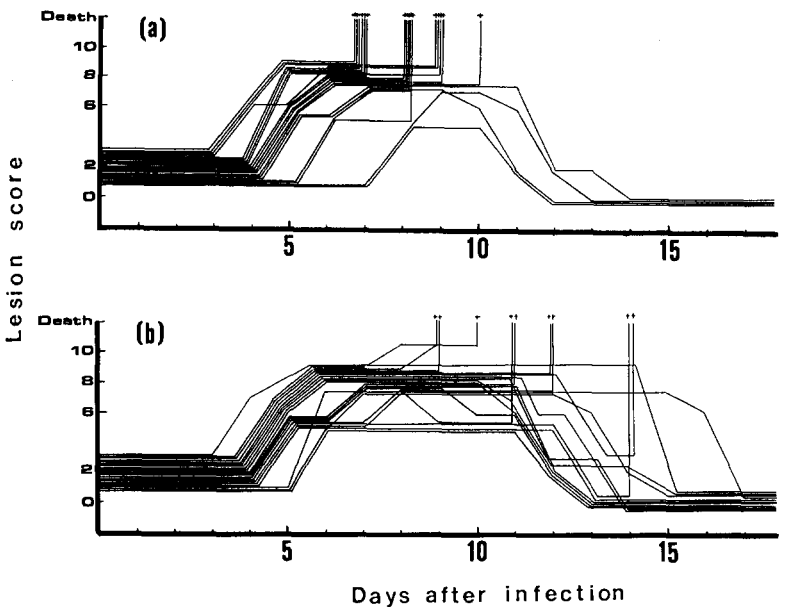

Fig. 1. Effect of IFN on the development of skin lesions in euthymic BALB/c mice inoculated intradermally with HSV-1. (a) Control mice; (b) IFN-treated mice.

Results

Protective effect of rMuIFN-β in euthymic BALB/c mice

In the first experiment, the protective effect of IFN was studied in euthymic BALB/c mice (20 per group). Treatment with IFN was initiated at 3 h after infection. IFN (0.08 ml, 8×10^5 U/mouse) was administered intraperitoneally (i.p.) once a day for 10 successive days, while mice given 0.15 M NaCl instead of IFN served as controls. Fig. 1 shows the time course of the development of skin lesions. In the control group, all mice developed zosteriform skin lesions by day 4, and almost all mice finally died. In the IFN-treated group, the development of zosteriform lesions was not significantly different from that of the control mice, but the mortality was significantly reduced ($p < 0.05$) and half of the mice survived through the 50-day observation period. Even for the dead mice, the survival time of IFN-treated mice was significantly prolonged ($P < 0.01$), the survival time for the control and IFN-treated mice being 8.0 ± 1.0 and 11.3 ± 1.9 days, respectively.

Effect of rMuIFN-β on virus growth in euthymic BALB/c mouse tissues

The virus titers of tissue homogenates of the skin, dorsal root ganglia and brain from each group of mice at various intervals after infection are shown in Fig. 2. In the control mice the virus titer in the skin rose as the skin lesions evolved to zosteriform lesions 5 days postinfection, and decreased when specific immunity was acquired, i.e. 7 days postinfection. The virus could be recovered from the homog-

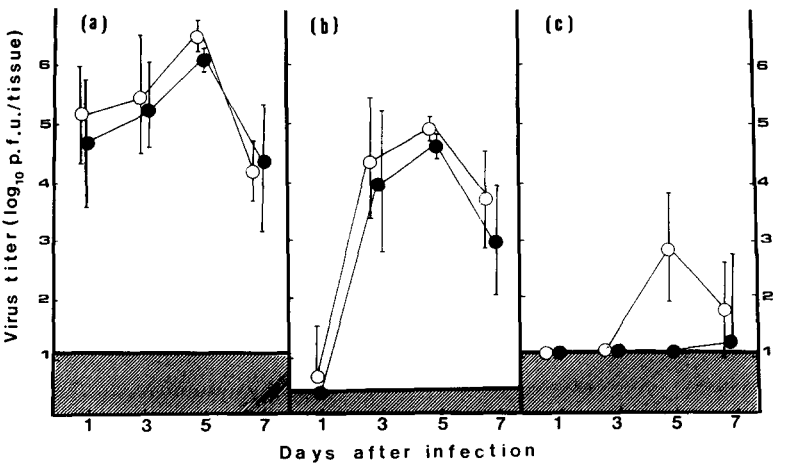

Fig. 2. Effect of IFN on virus growth in tissues of euthymic mice inoculated intradermally with HSV-1. At various intervals after infection, three mice from each group were sacrificed and the virus titers in their tissues were determined. (a) Skin (the minimum area encompassing the inoculated site and the whole lesion); (b) dorsal root ganglia; (c) brain. Symbols: ○, control mice; ●, IFN-treated mice. The hatched portion of each panel indicates the threshold of virus detection.

enates of dorsal root ganglia as early as 3 days after infection. In the brain, the virus was first recovered 5 days after infection.

In IFN-treated mice, the virus titer in the skin and dorsal root ganglia was slightly lower than that of control mice, though the difference was not significant. In the brain, however, the difference was significant ($p < 0.05$); at day 5 postinfection, when virus could not be detected in IFN-treated group, the virus titer reached its maximum in the control group.

Effect of the combination of rMuIFN-β and antibody in euthymic BALB/c mice

A protective effect of IFN was shown in the above experiments. However, since 45% of the IFN-treated mice finally died, the use of IFN and antibody in combination was tried, to achieve further antiviral effect. In this experiment, IFN and/or antibody treatment was initiated 3 h after infection. IFN (0.08 ml) was administered i.p. once a day for 10 successive days, and 0.5 ml of antibody was injected i.p. only once, 3 h after infection. The dose response was also studied.

The results are summarized in Table 1. In the control group, all mice developed zosteriform skin lesions beginning on day 4, and almost all mice died, as observed in the first experiment. Administration of 1:10 diluted IFN (8×10^4 U/mouse) as well as undiluted IFN (8×10^5 U/mouse) lowered the mortality to half of that of the control group. However, a lower dose of IFN (8×10^3 U/mouse) alone was

TABLE 1

Protective effect of rMuIFN-β and antibody in euthymic BALB/c mice infected with HSV-1.

Treatment[a]	Development of zosteriform skin lesions[b]		Mortality[c]		Latency[d]	
	No.	%	No.	%	No.	%
Control	40/40	(100)	36/40	(90)	3/4	(75)
IFN (8×10^5U[e])	20/20	(100)	9/20	(45)	11/11	(100)
IFN (8×10^4U)	10/10	(100)	4/10	(40)	6/6	(100)
IFN (8×10^3U)	10/10	(100)	10/10	(100)		
Ab (1:16[f])	7/10	(70)	2/10	(20)	6/8	(75)
Ab (1:1.6)	10/10	(100)	7/10	(70)	3/3	(100)
Ab (1:0.16)	9/9	(100)	9/9	(100)		
IFN (8×10^5U) + Ab (1:16)	4/10	(40)	0/10	(0)	6/10	(60)
IFN (8×10^5U) + Ab (1:1.6)	10/10	(100)	0/10	(0)	10/10	(100)
IFN (8×10^5U) + Ab (1:0.16)	10/10	(100)	4/10	(40)	5/6	(83)
IFN (8×10^4U) + Ab (1:1.6)	10/10	(100)	0/10	(0)	9/10	(90)
IFN (8×10^3U) + Ab (1:1.6)	9/10	(90)	5/10	(50)	3/4	(75)

[a] IFN and/or antibody (Ab) treatment was initiated at 3 h after infection.
[b] Number of mice with zosteriform skin lesions/number of mice tested (%).
[c] Number of dead mice / number of mice tested (%).
[d] Number of mice with infected ganglia / number of mice tested (%).
[e] A dose of 8×10^5 Units per mouse given once a day for 10 successive days.
[f] 0.5 ml of Ab (NT, 1:16) per mouse given only once.

TABLE 2

Therapeutic effect of rMuIFN-β and antibody in euthymic BALB/c mice infected with HSV-1.

Treatment[a]	Mortality[b]		Mean survival time (days + SD)
	No.	%	
Control	7/8	(88)	8.1 ± 0.69
IFN[c]	8/8	(100)	8.6 ± 1.60
Ab[d]	9/9	(100)	8.9 ± 1.17
IFN[c] + Ab[d]	7/8	(88)	8.4 ± 0.98

[a] IFN and/or antibody (Ab) treatment was initiated at 5 days after infection.
[b] Number of mice dead/number of mice treated (%).
[c] IFN (8×10^5) Units/mouse) was administered i.p. once a day for 10 successive days.
[d] 0.5 ml of Ab (NT, 1:16) was administered i.p. only once.

not effective; all mice developed zosteriform skin lesions and all died. Since the administration of rMuIFN-β alone reduced the mortality by approximately 50% at most, the combination of IFN and anti-HSV antibody was tried.

The use of IFN (8×10^5 U/mouse, 10 successive days) combined with only a single dose of undiluted human serum antibody (NT, 1:16) 3 h after virus challenge resulted in marked suppression of the development of skin lesions, i.e., only 4 of 10 mice developed slight zosteriform skin lesions and all mice survived, while after administration of undiluted human serum antibody alone 7 of 10 mice developed the zosteriform skin lesions and 2 of the 10 mice died. The combination was still effective when both IFN and antibody were diluted 1:10 but not when diluted 1:100.

Effect of rMuIFN-β treatment on the establishment of latent infection

In order to determine if the IFN treatment could affect the establishment of latency of the infecting HSV, the dorsal root ganglia were removed from the mice that survived 50 days or longer after infection and cocultivated with Vero cells. As shown in Table 1, there were no significant differences within each group in the number of mice in which latent infection was detected, though in mice treated with high-titer IFN (8×10^5 U/mouse) and antibody (NT, 1:16) the rate of latency was slightly lower than in the other groups.

Therapeutic effect of rMuIFN-β and antibody in euthymic BALB/c mice

Since a sufficient protective effect was obtained by administration of IFN and antibody, the therapeutic effect of the combination of IFN and antibody was determined in BALB/c mice. Treatment with IFN and antibody was initiated 5 days postinfection, when zosteriform skin lesions began to develop. IFN (8×10^5 U/mouse) was administered daily for 10 successive days and 0.5 ml of undiluted antibody (NT, 1:16) was administered once, together with the first injection of IFN.

The results shown in Table 2 indicate that neither suppression of further devel-

TABLE 3

Effect of combination of rMuIFN-β and antibody in athymic nude mice infected with HSV-1.

Treatment[a]	Mortality[b]		Mean survival time (days ± SD)
	No.	%	
Control	10/10	(100)	9.2 ± 0.9
IFN[c]	9/9	(100)	11.5 ± 2.3
Ab[d]	10/10	(100)	12.4 ± 2.2
IFN[c] + Ab[d]	10/10	(100)	16.5 ± 3.3

[a] IFN and/or antibody (Ab) treatment was initiated at 3 h after infection.
[b] Number of mice dead/number of mice treated (%).
[c] IFN (8×10^5 Units/mouse) was administered i.p. once a day for 10 successive days.
[d] 0.5 ml of Ab (NT, 1:16) was administered i.p. only once.

opment of skin lesions nor reduction of mortality was obtained when treatment with IFN and antibody was initiated 5 days after injection of virus. Once zosteriform skin lesions had developed, no therapeutic effect could be achieved.

Effect of the combination of rMuIFN-β and antibody in athymic nude mice

The effect of IFN in combination with antibody was studied by using athymic nude mice, an animal model of an immunocompromised host. Treatment was initiated 3 h postinfection. IFN (8×10^5 U/mouse) was administered i.p. once a day for 10 successive days and 0.5 ml of undiluted antibody (NT, 1:16) was administered i.p. only once 3 h postinfection.

As shown in Fig. 3 and summarized in Table 3, the survival time was significantly prolonged ($p < 0.01$) in mice treated with IFN alone, as compared with the control mice. The combination of IFN and antibody was more effective, allowing longer survival ($p < 0.01$) than IFN or antibody alone, though the mortality was not decreased.

TABLE 4

Effect of combination of rMuIFN-β and ACV in athymic nude mice infected with HSV-1.

Treatment[a]	Mortality[b]		Mean survival time (days ± SD)
	No.	%	
Control	8/8	(100)	9.1 ± 1.6
ACV[c]	8/8	(100)	18.6 ± 5.0
IFN[d] + ACV[c]	8/8	(100)	20.5 ± 5.0

[a] Treatment was initiated at 3 h after infection.
[b] Number of mice dead/number of mice treated (%).
[c] ACV (25 mg/kg of body weight) was administered i.p. every 12 h for 10 successive days.
[d] IFN (8×10^5 Units/mouse) was administered i.p. once a day for 10 successive days.

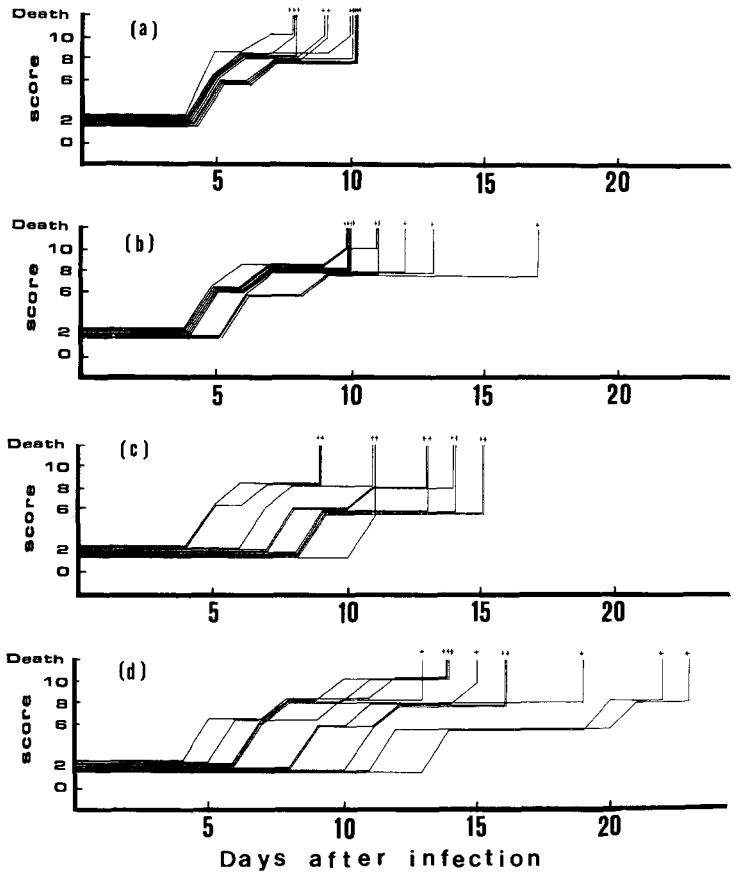

Fig. 3. Effect of IFN and antibody on the development of skin lesions of athymic nude mice inoculated intradermally with HSV-1. (a) Control mice; (b) IFN treated mice. IFN (0.08 ml, 8×10^5 U/mouse) was administered i.p. once a day beginning at 3 h after infection for 10 successive days; (c) antibody-treated mice. Five-tenths ml of antibody with a titer of 1:16 against HSV-1 was administered i.p. only once at 3 h after infection; (d) IFN- plus antibody-treated mice. IFN (0.08 ml 8×10^5 U/mouse) was administered i.p. beginning at 3 h after infection for 10 successive days and 0.5 ml of antibody (NT, 1:16) was administered i.p. only once at 3 h after infection.

Effect of the combination of rMuIFN-β and ACV in athymic nude mice

Finally, we investigated whether IFN could potentiate the protective effect of ACV in athymic nude mice. Treatment was initiated 3 h postinfection. IFN (8×10^5 U/mouse) was administered i.p. once a day for 10 successive days and ACV was administered i.p. (25 mg/kg of body weight) every 12 h for 10 successive days.

As shown in Table 4, ACV was effective when given alone and the survival time was significantly ($p < 0.01$) prolonged as compared with the control, though the mortality was not reduced. However, the effect of ACV was not significantly enhanced by the addition of IFN.

Discussion

There are a number of reports on the efficacy of IFN in HSV-infected mice. Endogenous IFN produced during experimental HSV infection is largely responsible for eventual spontaneous cures [10]. Zawatzky et al. [31] reported that mice susceptible to HSV infection, such as BALB/c mice, are low producers of IFN in response to HSV as compared with resistant C57BL/6 mice. They suggested that the magnitude of early IFN responses upon HSV infection correlated with resistance. Their subsequent experiments [30] demonstrated that i.p. inoculation of 250 LD_{50} of HSV resulted in a high titer of IFN in the peritoneal cavity of C57BL/6 mice followed by the survival of almost all mice. On the other hand, injections of 25 or 2.5 LD_{50} of HSV did not induce measurable amounts of IFN.

In our laboratory, preliminary experiments indicated that mouse embryo fibroblast cells pretreated with rMuIFN-β were protected against subsequent challenge with HSV-1. The present studies were then designed to determine whether exogenous IFN exerted a favorable effect on HSV-infection in BALB/c mice.

In euthymic mice infected intradermally with HSV-1, viral replication was detectable 3 days after infection in the dorsal root ganglia. Subsequently, all mice developed zosteriform skin lesions and almost all mice died at or prior to day 10 due to encephalitis, as evidenced by limb paralysis and virus growth in the brain. It has been reported that when mice are given a single large dose of IFN i.p., serum IFN levels peak at about 1 h postinjection and are virtually reduced to zero after 8 h [13]. HSV-infected mice were treated i.p. with rMuIFN-β (8×10^5 U/dose) for 10 successive days. In IFN-treated mice, the development of zosteriform skin lesions was not significantly different from that of control mice, but the mortality was significantly reduced. Viral replication in the brain was not detectable in IFN-treated mice at 5 days after infection, at which time the virus titer was maximum in the control-infected mice. From the results it was inferred that rMuIFN-β administered i.p. did not significantly inhibit virus growth at the site of inoculation or prevent invasion of the central nervous system, but exerted an effect on the outcome of the infection by inhibiting virus replication in the brain. In fact, when the treatment was started at day 5 after infection, the IFN did not protect the mice any longer. The same inference was also made from the experiment using athymic nude mice, where rMuIFN-β prolonged the survival time, though it failed to reduce the mortality.

The mechanism(s) by which IFN inhibits HSV infection are poorly understood [2,7]. The antiviral mechanism of IFN in vivo is considered to be due in part to direct induction of an antiviral state in the host cells. Kirchner et al. [16] suggested that in vivo IFN would predominantly exert a direct antiviral effect on the target cells infected by HSV. Especially, their data stress the important role of the IFN produced at the site of infection. On the other hand, Kohl [17] reported that whereas recombinant human IFN-α or human leukocytes alone did not protect mice from lethal challenge with HSV, a combination of them afforded significant protection. He speculated that the mechanism of this protection probably involved in vivo stimulation of human natural killer cytotoxicity by IFN. It is well known that

IFN indirectly suppresses virus infection by enhancement of the host immune system [6,19]. IFNs enhance natural killer cell activity, T-cell-mediated cellular cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) [12,23, 25,29]. Since there is evidence that it is rather difficult for exogenous interferon to gain access to some organs, in particular the brain [13], we favor the interpretation that rMuIFN-β suppresses virus growth in the brain indirectly by enhancing the host immune system.

We also studied the combined effect of IFN and antibody. The treatment by a combination of rMuIFN-β and heterologous antiserum resulted in a stronger antiviral effect than was achieved with either agent used alone. Although the establishment of latent infection was not completely inhibited, the combined treatment appeared to be very effective in limiting the extent of subsequent tissue involvement (data not shown). In the experiment using athymic nude mice, the animals finally died even if they were treated with antibody or ACV combined with IFN. However significant increase in survival of antibody-treated mice was obtained by the addition of IFN. We suggested previously, on the basis of experiments with athymic nude mice, that ADCC might play an important role in protection from HSV infection [11,15,28]. It has also been shown that IFN augments ADCC in vitro [12]. Accordingly, the present findings show that IFN might augment ADCC in vivo.

Herpesvirus encephalitis is now regarded as the most frequently fatal endemic virus infection of the central nervous system. The morbidity and mortality associated with herpesvirus infections is markedly enhanced in immunosuppressed patients. Thus, herpesviruses have become prime targets for prospective therapeutic agents. Although we could not clearly determine the mechanisms of the antiherpetic effect of rMuIFN-β, it protected the mice from HSV infection and its effect was enhanced by antibody. Therefore, the possibility of the clinical use of recombinant human IFN-β, either singly or combined with antibody, against HSV infection should be considered.

Acknowledgements

The authors are grateful to Basic Research Laboratories, Toray Industries Inc., for providing the recombinant murine interferon-β, and to Dr. Mary Louise Robbins for reviewing the manuscript.

References

1 Cerruti, R.L., Connell, E.V., Trown, P.W. and Sim, S. (1985) Synergistic interaction between interferon-α and acyclovir in the treatment of herpes simplex virus type 1 infection in mice. Antiviral Res. Suppl. 1, 217–223.

2 Chatterjee, S., Hunter, E. and Whitley, R. (1985) Effect of cloned human interferons on protein synthesis and morphogenesis of herpes simplex virus. J. Virol. 56, 419–425.

3 Czarniecki, C.W., Fennie, C.W., Powers, D.B. and Estell, D.A. (1984) Synergistic antiviral and antiproliferative activities of *Escherichia coli*-derived human alpha, beta, and gamma interferons. J. Virol. 49, 490–496.
4 De Clercq, E. and Luczak, M. (1976) Intranasal challenge of mice with herpes simplex virus: An experimental model for evaluation of the efficacy of antiviral drugs. J. Infect. Dis. 133 (Suppl.), A226–236.
5 De Clercq, E., Zhen-Xi, Z., Descamps, J. and Huygen, K. (1981) *E*-5-(2-bromovinyl)-2′-deoxyuridine vs. interferon in the systemic treatment of infection with herpes simplex virus of athymic nude mice. J. Infect. Dis. 143, 846–852.
6 De Maeyer, E. and De Maeyer-Guignard, J. (1980) Immunoregulatory action of type I interferon in the mouse. Ann. N.Y. Acad. Sci. 350, 1–11.
7 Esteban, M. and Paez, E. (1985) Antiviral and antiproliferative properties of interferons: Mechanism of action. Prog. Med. Virol. 32, 159–173.
8 Fish, E.M., Banerjee, K. and Stebbing, N. (1983) Antiviral activities of cloned human leukocyte interferons against herpes simplex virus type 2 infection of mice. J. Gen. Virol. 64, 2317–2321.
9 Fraser-Smith, E.B., Eppstein, D.A., Marsh, Y.V. and Matthews, T.R. (1985) Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl) guanine in combination with gamma interferon against herpes simplex virus type 2 in mice. Antiviral Res. 5, 137–144.
10 Gresser, I., Tovey, M.G. Maury, C. and Bandu, M.T. (1976) Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza viruses. J. Exp. Med. 144, 1316–1323.
11 Hayashida, I., Nagafuchi, S., Hayashi, Y., Kino, Y., Mori, R., Oda, H., Ohtomo, N. and Tashiro, A. (1982) Mechanism of antibody-mediated protection against herpes simplex virus infection in athymic nude mice: Requirement of Fc portion of antibody. Microbiol. Immunol. 26, 497–509.
12 Herberman, R.R., Ortaldo, J.R. and Bonnard, G.D. (1979) Augmentation by interferon of human natural and antibody-dependent cell-mediated cytotoxicity. Nature 277, 221–223.
13 Heremans, H., Billiau, A. and De Somer, P. (1980) Interferon in experimental viral infections in mice: Tissue interferon levels resulting from the virus infection and from exogenous interferon therapy. Infect. Immun. 30, 513–522.
14 Janz, C. and Wigand, R. (1982) Combined interaction of antiherpes substances and interferon on the multiplication of herpes simplex virus. Arch. Virol. 73, 135–143.
15 Kino, Y., Hayashi, Y., Hayashida, I. and Mori, R. (1980) Dissemination of herpes simplex virus in nude mice after intracutaneous inoculation and effect of antibody on the course of infection. J. Gen. Virol. 63, 475–479.
16 Kirchner, H., Zawatzky, R., Storch, E., Domke, I. and Chmielarczyk, W. (1985) On the role of endogenous interferon in natural and induced antiviral resistance. Antiviral Res. Suppl. 1, 155–159.
17 Kohl, S. (1983) Stimulation of human natural killer cytotoxicity and protection of mice from infection due to herpes simplex virus by recombinant human leukocyte interferon. J. Infect. Dis. 147, 484–488.
18 Langford, M.P., Villarreal, A.L. and Stanton, G.J. (1983) Antibody and interferon act synergistically to inhibit enterovirus, adenovirus and herpes simplex virus infection. Infect. Immun. 41, 214–218.
19 Lebel, F. and Hirsch, M.S. (1985) The role of interferon in immunity and prophylaxis. In: The Herpesviruses, vol. 4 (Roizman, B. and Lopez, C., eds.). pp. 371–393. Plenum Press, New York.
20 Moran, D.M., Kern, E.R. and Overall, J.C. Jr. (1985) Synergism between recombinant human interferon and nucleoside antiviral agents against herpes simplex virus: Examination with an automated microtiter plate assay. J. Infect. Dis. 151, 1116–1122.
21 Nagafuchi, S., Oda, H., Mori, R. and Taniguchi, T. (1979) Mechanism of acquired resistance to herpes simplex virus infection as studied in nude mice. J. Gen. Virol. 44, 715–723.
22 Olsen, G.A., Kern, E.R., Overall, J.C. Jr. and Glasgow, L.A. (1978) Effect of treatment with exogenous interferon, polyriboinosinic-polyribocytidylic acid, or polyriboinosinic-polyribocytidylic acid-poly-L-lysine complex on herpesvirus hominis infections in mice. J. Infect. Dis. 137, 428–436.
23 Ortaldo, J.R., Mason, A., Rehberg, E., Moschera, J., Kelder, B., Pestka, S. and Herberman, B.

(1983) Effects of recombinant and hybrid recombinant human leukocyte interferons on cytotoxic activity of natural killer cells. J. Biol. Chem. 258, 15011–15015.

24 Simmons, A. and Nash, A.A. (1984) Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. J. Virol. 52, 816–821.

25 Stanwick, T.L., Campbell, D.E. and Nahmias, A.J. (1980) Spontaneous cytotoxicity mediated by human monocyte-macrophage against human fibroblasts infected with herpes simplex virus-Augmentation by interferon. Cell. Immunol. 53, 413–416.

26 Worthington, M.G., Conliffe, M. and Williams, J. (1977) Treatment of fatal disseminated herpes simplex virus, type 1, infection in immunosuppressed mice. Proc. Soc. Exp. Biol. Med. 156, 168–172.

27 Yamamoto, M., Hayashi, Y., Tang, J.-L. and Mori, R. (1985) Effects of combined use of acyclovir and antibody in athymic nude mice inoculated intracutaneously with herpes simplex virus. Antiviral Res. 5, 83–91.

28 Yamamoto, M., Tang, J.-L., Kumano, Y., Mori, R. and Kino, Y. (1986) Ability of monoclonal antibody to herpes simplex virus glycoprotein gB to promote healing of herpetic skin lesions in nude mice. Antiviral Res. 6, 223–231.

29 Zarling, J.M., Sosman, J., Eskra, L., Borden, E.C., Horoszewicz, J.S. and Carter, W.A. (1978) Enhancement of T cell cytotoxic responses by purified human fibroblast interferon. J. Immunol. 121, 2002–2004.

30 Zawatzky, R., Gresser, I., DeMaeyer, E. and Kirchner, H. (1982) The role of interferon in the resistance of C57BL/6 mice to various doses of herpes simplex virus type 1. J. Infect. Dis. 146, 405–410.

31 Zawatzky, R., Hilfenhaus, J., Marcucci, F. and Kirchner, H. (1981) Experimental infection of inbred mice with herpes simplex virus type 1. I. Investigation of humoral and cellular immunity and of interferon induction. J. Gen. Virol. 53, 31–38.