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Antiviral effects of recombinant human tumor necrosis factor-alpha in combination with natural interferon-beta in mice infected with herpes simplex virus type 1

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Summary

The protective effects of combination therapy utilizing recombinant human TNF-alpha (rTNF-α) and natural murine interferon-beta (IFN-β) in mice infected with herpes simplex virus type 1 (HSV-1) was investigated. Mice treated with rTNF-α alone at all of the doses tested (a single i.v. administration, 2.3–2,300 μg/kg; multiple i.p. administrations 0.4–250 μg/kg) as well as mice that received IFN-β alone at doses of 16×10^4 U/kg or less resulted in a 0% survival rate. Combination therapy consisting of a single administration of rTNF- α (230 and 23 μg/kg) and multiple administrations of IFN-β (4×10^4 U/kg) resulted in a 40% and 60% survival rate. Multiple treatments of infected mice with rTNF-α (50 and 10 μg/kg) in combination with IFN-β (4×10^4 U/kg) resulted in 50% and 70% survival rates, respectively. These results suggest that the combination therapy of rTNF and natural murine IFN-β produce synergistic protective effects in mice infected with a lethal amount of HSV-1.

Synergism; Antiviral effect; Human recombinant TNF-alpha; Natural murine IFN-beta

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Tumor necrosis factor (TNF), a protein consisting of 157 amino acids (Aggarwal et al., 1985), has been shown to demonstrate antiviral activity in vivo and in vitro (Goeddel et al., 1986). TNF has demonstrated its in vitro antiviral activity in three different ways. First, TNF has demonstrated the ability to selectively lyse virus-infected cells while having no effect on normal cells (Aderka et al., 1985). A second way that TNF expresses its antiviral activity is that TNF has been shown to protect cells from virus infection and reduce the yield of virus progeny in an interferon-like manner (Ruggiero et al., 1989; Mestan et al., 1986). A third method in which TNF can express its antiviral activity has been reported by Wong and Goeddel (Wong and Goeddel, 1986). They have shown that TNF inhibits virus replication and protects cells from lysis without an apparent contribution from endogenous interferon. This antiviral activity was strongly synergistic with IFN-α and IFN-γ (Feduchi et al., 1989; Mestan et al., 1988; Wietzerbin et al., 1990). The antiviral activity of TNF was also demonstrated in an in vivo system. Rossol-Voth et al. (Rossol-Voth et al., 1991) demonstrated that TNF protected C57BL/6 mice from a lethal infection with HSV-1. In their experiments, they were unable to determine the exact mechanism of this antiviral activity of TNF.

In addition to direct protective antiviral effects, TNF has also exhibited various immunomodulatory activities (Phillip and Epstein, 1986). These immunomodulatory activities may play an important role when TNF exhibits antiviral activities in vivo. Interferon-beta (IFN-β) is a substance known to possess antiviral activities. The antiviral activity of IFN-β can either be expressed as a direct protective effect of cells or through its immunomodulatory effects (Grossberg, 1987). Therefore, this report will describe the effects of combination therapy using rTNF-α and IFN-β on the survival of mice infected with a lethal amount of HSV-1.

rTNF-α used in these experiments was provided by Knoll Pharmaceuticals (Whippany, NJ) in a lyophilized form (specific activity = 5×10^6 U/mg protein). Natural IFN-β was a gift from Dr. Kobayashi of the Basic Research Laboratory, Toray Institute, Tokyo, Japan. It was induced by stimulation of L-MS cells with Newcastle disease virus, as described previously (Saito et al., 1983). The antiviral titer of this preparation was 9.2×10^6 reference units per ml (specific activity: 6.7×10^6 reference units per mg of protein). The IFN activity was assayed by a 50% plaque reduction method using L-Galveston cells infected with VSV as described previously (Rubinstein, 1981). Vero cells and L-Galveston cells were grown in Eagle's Minimum Essential Medium (EMEM) containing 10% fetal calf serum (FCS), 1% L-glutamine, penicillin and streptomycin (growth medium). The KOS strain of HSV-1 that was used in these experiments was propagated in Vero cells and stored at $-70°C$ until use. The titer of this virus was 1.8×10^7 PFU/ml as titered by plaque assay on Vero cells cultured in maintenance media (EMEM supplemented with 2% FCS, 1% L-glutamine, penicillin and streptomycin). Seven-week-old male BALB/c mice (Jackson Laboratories, Bar Harbor, Maine) were used in these experiments. The mice were inoculated i.p. with HSV-1 at a concentration of 10 LD_{50}. A

dose of 4×10^2 PFU/mouse was required to kill 50% of the mice in our experimental system. HSV-infected mice were treated with rTNF-α and/or IFN-β using the following treatment regimens: (a) saline; (b) a single administration of rTNF-α (i.v., 2.3–2,300 μg/kg) one day before HSV-1 infection; (c) multiple administrations of rTNF-α (i.p., 2–250 μg/kg) beginning one day before HSV-1 infection and continuing for a total of 10 treatments; (d) multiple administrations of IFN-β (i.p., $1–256 \times 10^4$ U/kg) beginning one day before HSV-1 infection and continuing daily for a total of 10 treatments; (e) a single i.v. administration of rTNF-α (230 or 23 μg/kg) plus multiple i.p. administrations of IFN-β (4×10^4 U/kg); (f) multiple i.p. administrations of rTNF-α (50 or 10 μg/kg) plus multiple administrations of IFN-β (4×10^4 U/kg). In the combination experiments, a single i.v. dose of 2,300 μg/kg or multiple administrations of 250 μg/kg of rTNF-α was not used because these doses caused weight loss and poor general appearance in mice. The single i.v. administration was performed by injection into the tail vein of mice one day before infection, while the multiple i.p. administrations began 1 day before infection and continued daily for a total of 10 treatments. The antiviral effect of the two agents, both alone and in the various combination therapies, was evaluated on the basis of survival rate and mean survival days (MSD). Fifteen days after virus infection, the survival rate was calculated. MSD was calculated as the average number of days the mice in each treatment group survived. The significance of the results was analyzed using the x^2-test (% survival) and Student's *t*-test (MSD). The results of the % survival in the combination therapies were compared to saline-treated mice, because the mice in this group as well as those treated with either rTNF-α alone or IFN-β all died. However, when the significance of the MSD was analyzed, the combination therapy results were compared to the results of the mice treated with saline, rTNF-α, or IFN-β alone.

The survival rate of HSV-infected mice that were treated with rTNF-α alone was not changed as compared with infected mice treated with saline. The results obtained from mice which received either a single i.v. administration or multiple i.p. administrations of rTNF-α revealed that no antiviral effect was demonstrated at any of the concentrations (i.v. = 2.3 to 2,300 μg/kg; i.p. = 0.4 to 250 μg/kg) used in this experiment. These results indicated that rTNF-α alone could not protect the mice from a lethal infection of HSV-1. As shown in Table 1, HSV-infected mice received multiple administrations of IFN-β in doses ranging from 1×10^4 to 256×10^4 U/kg. Doses of 64×10^4 U/kg or higher protected the mice (100% survival) from the lethal effects of the HSV-1 infection, while doses of 16×10^4 U/kg or lower did not significantly alter the effects of the HSV-1 infection. These results indicated that high doses (64×10^4 U/kg or more) of IFN-β inhibited the infection of HSV-1 and lower doses have no effect. On the basis of these results, we chose a 4×10^4 U/kg dose of IFN-β for the combination therapy because this dose by itself did not have any antiviral effect against the HSV-1 infection. In the next set of experiments, the effect of combination therapy using IFN-β and a single i.v. administration of

TABLE 1

Protective effect of IFN-β on HSV infection in mice

Dose[a] ($\times 10^4$ U/kg)	Number of Mice	Mean survival days[b]	Survivors treated (%)
256	10	>15.0[c]	10/10 (100)[d]
64	10	>15.0[c]	10/10 (100)[d]
16	10	8.5	0/10
4	10	8.9	0/10
1	10	8.5	0/10
Saline	20	8.4	0/20

[a]Mice infected with a 10 LD_{50} dose of HSV-1 were treated with various doses of murine natural inteferon-beta (IFN-β) i.p. once a day for 10 days beginning 1 day before the infection;
[b]Survival of mice was observed for an experimental period of 15 days;
[c]Students t-test, $P < 0.001$;
[d]χ^2 analysis, $P < 0.001$.

rTNF-α was evaluated in HSV-infected mice. HSV-infected mice treated with IFN-β and a single i.v. administration of rTNF-α (230 μg/kg) resulted in a survival rate of 40% ($P < 0.01$), while HSV-infected mice treated with a single i.v. administration of rTNF-α (23 μg/kg) in combination with IFN-β demonstrated a 60% ($P < 0.01$) survival rate (Table 2). Multiple i.p. administrations of TNF-α (50 and 10 μg/kg) in combination with IFN-β also protected mice from HSV-1 infections. HSV-infected mice treated with a combination of rTNF-α (50 μg/kg) and IFN-β demonstrated a survival rate of 50% ($P < 0.01$) over the 15-day observation period. When multiple doses of 10 μg/kg of rTNF-α was combined with IFN-β, 70% ($P < 0.01$) of the infected mice in this group survived as compared to infected mice treated with saline. These results indicate that combination therapy using a single or multiple administrations of rTNF-α and multiple administrations of IFN-β protect mice from death by HSV-1 infection.

The results obtained in our experiments show that rTNF-α and IFN-β exhibit synergistic effects in protecting mice against HSV-1 infection. As shown above, rTNF-α alone was incapable of protecting mice from HSV-1 infection at any of the doses assayed. Doses of IFN-β greater than 16×10^4 U/kg also did protect the mice, but doses of 16×10^4 U/kg or less did not protect the mice against HSV-1 infection. Therefore, a dose of 4×10^4 U/kg of IFN-β was chosen as the dosage for the combination therapy. The doses of rTNF-α (230 and 23 μg/kg for the single i.v. administration; 50 and 10 μg/kg for the multiple i.p. administrations) were chosen because there was no toxicity such as weight loss seen in normal mice treated with these doses. Combination therapy using IFN-β and a single i.v. administration of rTNF-α resulted in a 40% survival rate when a dose of 230 μg/kg of rTNF-α was used, while the survival rate was 60% when a dose of 23 μg/kg of rTNF-α was used. Multiple i.p. administrations of rTNF-α at doses of 50 and 10 μg/kg resulted in a 50% and 70% survival rates, respectively, when they were administered in combination with IFN- β to HSV-infected mice. These results demonstrate

TABLE 2

Survival of HSV-1-infected mice treated with rTNF-α and IFN-β in combination

Mice were treated with:[a]	Number of mice	Mean Survival Days[b]	Survivors treated (%)
A. Single administration of rTNF-α[c]			
Saline	20	7.4	0/20
IFN-β alone	20	8.7	0.20
rTNF-α alone, 230 μg/kg	10	8.2	0/10
rTNF-α alone, 23 μg/kg	10	8.2	0.10
rTNF-α , 230 μg/kg + IFN-β	10	>11.0[e]	4/10 (40)[f]
rTNF-α , 23 μg/kg + IFN-β	10	>12.7[e]	6/10 (60)[f]
B. Multiple administrations of rTNF-α[d]			
Saline	20	8.1	0/20
IFN-β alone	20	8.9	0/20
rTNF-α alone, 50 μg/kg	10	8.8	0/10
rTNF-α alone, 10 μg/kg	10	8.7	0/10
rTNF-α , 50 μg/kg + IFN-β	10	>11.2	5/10 (50)
rTNF-α , 10 μg/kg + IFN-β	10	>13.3	7/10 (70)

[a]Mice infected with a 10 LD_{50} dose of HSV-1 were treated with saline, IFN-β alone, a single or multiple doses of rTNF-α alone, or their combinations. IFN-β was administered i.p. to mice beginning one day before HSV-1 infection and continuing daily for a total of 10 treatments at a dose of 4×10^4 U/kg;

[b]The mean survival days and % survival were calculated by observation of the mice over a period of 15 days;

[c]rTNF-α was administered i.v. one day before the HSV infection to mice;

[d]rTNF-α was administered i.p. beginning one day before HSV-1 infection and continuing once per day for a total of 10 treatments.

[e]Student's *t*-test, $P < 0.001$, as compared to the saline-treated mice (Comparison between IFN-β alone or rTNF-α alone of their combination was significant; $P < 0.001$).

[f]χ^2 analysis, $P < 0.001$, as compared to the saline-treated mice.

that the combination therapy may exert a synergistic effect on HSV-1 infection in mice. There have been several reports that TNF exerts its antiviral effect through induction of IFN-β. Reis et al. (Reis et al., 1988) demonstrated that addition of monoclonal antibody to IFN-β to TNF-treated cells abrogated the antiviral effect of TNF. Cells which received combination therapy with TNF and IFN-γ demonstrated antiviral activity against VSV. However, treatment of these cells with rabbit antibody to IFN-β resulted in a 99% inhibition of the antiviral effect of the combination therapy (Hughes et al., 1988). They offered additional evidence (Hughes et al., 1989) in that hydrazine sulfate, a non-competitive inhibitor of the enzyme phosphoenolpyruvate carboxykinase in gluconeogenesis, enhanced the antiviral activity of TNF. Hydrazine sulfate showed no antiviral activity itself and potentiated the antiviral action of TNF by enhancing the activity of IFN-β. These reports and our results presented here, which show that IFN-β and rTNF-α may express synergism, indicate that IFN-β plays an important role when rTNF-α expresses antiviral effects in tissue culture and animal models.

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