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SHORT COMMUNICATION

ACTIVITY OF RECOMBINANT HUMAN ALPHA INTERFERON AGAINST INFLUENZA VIRUS INFECTION IN MICE

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The in vivo antiviral activity of recombinant human leukocyte hybrid interferon, HuIFN-αAD, was examined.

Results showed that this material in highly purified form did not protect mice against a lethal dose of influenza virus, although administration of natural MuIFN-α/β to mice infected with a lethal dose of influenza virus had a marked protective effect.

The effect of alveolar macrophages treated with IFN on influenza virus replication was examined in vitro. The antiviral activity of alveolar macrophages treated with HuIFN-αAD was lower than that of MuIFN-α/β. It is concluded that HuIFN-αAD is effective in direct inhibition of influenza virus, but not in indirect inhibition mediated by alveolar macrophages or in protection of mice from influenza virus infection.

The construction of hybrid IFN genes by recombinant DNA technology has produced IFNs that have relatively high specific activities on human, bovine and murine cell lines (Streuli et al., 1981). The presence of common restriction endonuclease sites in the gene sequences of HuIFN-αA and HuIFN-αD (Goeddel et al., 1981) has facilitated the production of hybrid IFN designated as HuIFN-αAD. HuIFN-αAD induced marked antiviral activity in mouse cells, although natural and recombinant human IFN preparations did not show any appreciable activity in mouse cells. This hybrid HuIFN-αAD also protected mice against lethal doses of encephalomyocarditis virus infection (Weck et al., 1982).

In the present study, we examined the antiviral effect of recombinant HuIFNs against influenza virus infection of mice.

HuIFN-αAD with a specific activity of 1.5×10^7 IU per mg protein was supplied by the Central Research Division, Takeda Pharmaceutical Industries, Ltd., Japan. The con-

struction and expression of bacterial plasmids containing human IFN genes have been described previously (Goeddel et al., 1980; Goeddel et al., 1981).

Mouse IFN-α/β was prepared on mouse L929 cells, which were subcultured and maintained in Eagle minimum essential medium (EMEM) containing 5% fetal calf serum (FCS) as described by Knight (1977). The L929 cells were infected with Newcastle disease virus (NDV, Miyadera strain), and the culture fluid was harvested as crude IFN after incubation for 24 h at 37 C. This IFN preparation was partially purified by the method of Knight (1977). Namely, the crude preparation was dialyzed against distilled water, the precipitate produced by dialysis was removed by centrifugation, and the supernatant was lyophilized. The specific activity of MuIFN was 1.2×10^6 IU per mg protein.

IFN titers were determined in a cytopathic effect inhibition assay performed in microtiter plates using L929 or MDBK cells and vesicular stomatitis virus (VSV, New Jersey strain). The NIH standard for HuIFN or MuIFN was used in these assays, and all titers were expressed in international units (IU).

Mouse adapted influenza A virus (A/PR/8/34), which was propagated in chicken embryonated eggs, was used in this study. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench (1938).

Female ICR mice weighing 18 to 20 g, obtained from Charles River Inc., Japan, were exposed to nebulized influenza virus at 10 LD_{50} in an airborne infection apparatus for 30 min. Each experimental group consisted of 12 mice.

HuIFN-αAD was administered by intranasal instillation into mice under deep nembutal narcosis 24, 3 and 1 h before virus challenge, 1 and 3 h after virus challenge and then once daily for 6 days.

Intranasal injection of HuIFN-αAD at doses of 2×10^4 IU per animal daily from day -1 to 6 resulted in 10% protection. Even at doses of 1×10^5 IU, HuIFN-αAD had no significant protection against influenza virus infection (Fig. 1). Therefore, HuIFN-αAD was concluded not to affect influenza virus infection.

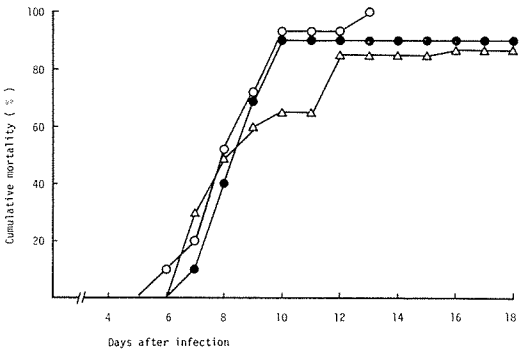

FIGURE 1. Protective effect of recombinant HuIFN-αAD on influenza virus infection in mice.
IFN or placebo was administered intranasally 24, 3 and 1 h before virus challenge (10 LD_{50}), 1 and 3 h after virus challenge and then once daily for 6 days. ●; 2×10^4 IU of HuIFN-αAD, △; 1×10^5 IU of HuIFN-αAD, ○; Placebo.

HuIFN-αA was also ineffective against influenza virus infection (data not shown).

The prophylactic effect of MuIFN-α/β on influenza virus infection in mice was examined. Mice were infected with a lethal does of influenza virus, and were treated intranasally daily from day -1 to 6 with 2×10^4 IU of MuIFN-α/β or placebo, which was calf serum diluted with saline to an equal protein concentration to that of MuIFN-α/β. The cumulative mortality in the IFN-treated group was lower than that in the placebo-treated, control group. As shown in Fig. 2, MuIFN-α/β protected about 60% of the mice inoculated with a lethal dose of virus. This means that MuIFN-α/β was significantly effective in protecting mice from influenza ($P<0.005$ by the χ^2 test).

The antiviral activity of HuIFN-αAD was compared with that of MuIFN-α/β in L929 cells. HuIFN-αAD showed marked antiviral activity against VSV infection in L929

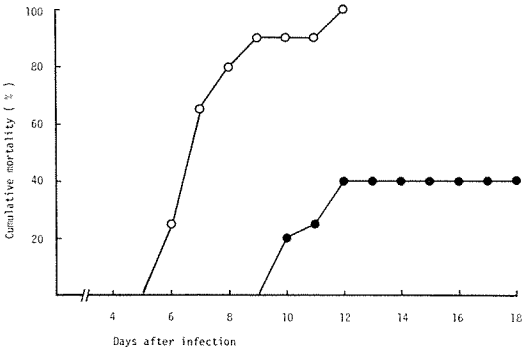

FIGURE 2. Protective effect of MuIFN-α/β on influenza virus infection in mice.

IFN or placebo was administered intranasally 24, 3 and 1 h before virus challenge (10 LD_{50}), 1 and 3 h after virus challenge and then once daily for 6 days. ●; 2×10^4 IU of MuIFN-α/β, ○; Placebo.

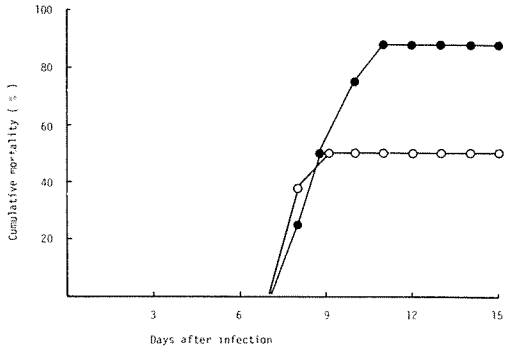

FIGURE 3. Role of alveolar macrophages in mice infected with influenza virus.

Cumulative mortality of mice given an intranasal injection of carrageenan (1 mg/mouse) on day 0 after virus infection (2 LD_{50}) (●). Control mice were treated with physiological saline (○).

cells, and the ratio of the antiviral activity of HuIFN-αAD to that of MuIFN-α/β in these cells was 1.2 (data not shown). However, in an in vivo experiment, there was a significant difference in the cumulative mortalities of HuIFN-αAD- and MuIFN-α/β-treated groups. This suggests that the protective effect of MuIFN-α/β against influenza infection in mice may not be related with direct inhibition of virus multiplication by IFN, but with indirect inhibition mediated by biological activities of IFN other than antiviral activity.

The role of alveolar macrophages in influenza virus infection was examined using carrageenan (Type V, Sigma Chemicals, Co., Ltd.), which is an anti-macrophage substance (Catanzaro, Schwartz and Graham, 1971). Carrageenan caused significant increase in the mortality of mice infected with virus at a dose of 2.0 LD_{50} when it was administered intranasally on day 0 at a dose of 1.0 mg per mouse. Of the infected mice, 87.5% treated with carrageenan died, compared with 50% of those that were not treated ($p<0.05$ by the χ^2 test) (Fig. 3). This suggests that macrophages are important in the pathogenesis of influenza virus infection.

Next, the effect of alveolar macrophages treated with HuIFN-αAD or MuIFN-α/β on influenza virus replication was examined in an in vitro system. Influenza virus was adsorbed to MDCK cells (1×10^6 cells per ml) in roller culture tubes at 10 $TCID_{50}$ of virus per tube for 1 h at 34 C. Unabsorbed virus were removed by washing. Then, alveolar macrophages (1×10^5 cells per tube), most of which were cells morphologically resembling macrophages, with or without IFN treatment were added to each tube. The supernatant fluid was harvested after incubation for 3 days and its virus titer was measured. Treatment of the macrophages with MuIFN-α/β (1×10^3 IU/ml) caused strong inhibition of virus replication. A similar experiment was done using HuIFN-αAD (1×10^3 IU/ml) cells, and results showed that the effect of HuIFN-αAD on antiviral activity was less than that of MuIFN-α/β (Table 1).

MuIFN-α/β enhanced the phagocytic activity of mouse peritoneal exudate cells, but HuIFN-αAD did not (personal communication). This means that mouse L929 cells are highly sensitive to HuIFN-αAD, but mouse macrophages are not.

The differences in the protective effects of

TABLE 1. *Comparison of antiviral activities of alveolar macrophages with and without IFN-treatment*[a]

Treatment	Virus yield ($TCID_{50}$/ml)		
	Exp. 1	Exp. 2	Mean
None	$10^{5.25}$	$10^{4.75}$	$10^{5.0}$
AM[b]	$10^{5.0}$	$10^{4.5}$	$10^{4.75}$
AM+α/β[c]	$10^{3.0}$	$10^{2.5}$	$10^{2.75}$
αAD	$10^{3.75}$	$10^{3.25}$	$10^{3.50}$
α/β	$10^{4.5}$	$10^{4.0}$	$10^{4.25}$
αAD[d]	$10^{5.0}$	$10^{4.5}$	$10^{4.75}$

[a] MDCK cells (1×10^6 cells) infected with 1.0 $TCID_{50}$ of influenza virus were overlaid with 1×10^5 alveolar macrophages with or without IFN-treatment, and the virus yield was measured 3 days later.
[b] AM: Alveolar macrophages
[c] α/β: MuIFN-α/β (1×10^3 IU/ml)
[d] αAD: HuIFN-αAD (1×10^3 IU/ml)

MuIFN-α/β and HuIFN-αAD on influenza virus infection in mice may be due to difference in the inhibitory effects on influenza virus replication of alveolar macrophages treated with HuIFN-αAD and with MuIFN-α/β, because alveolar macrophages may be important in the pathogenesis of influenza virus infection.

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