EFFECTS OF HUMAN α, β AND γ INTERFERONS ON VARICELLA ZOSTER VIRUS *IN VITRO*

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Abstract. The antiviral effects of interferon (IFN) on varicella zoster virus (VZV) and herpes simplex virus (HSV) *in vitro* were examined. The values for the 50% inhibitory dose (ID_{50}) of IFN-α, β and γ determined by plaque reduction assay, were 0.813, 0.650 and 13.750 IU/ml, respectively, against VZV and 18.00, 10.38 and 115.0 IU/ml, respectively, against HSV. Thus IFN-α and β were more effective than IFN-γ against both VZV and HSV and VZV was more sensitive than HSV to the IFNs. Five mutants of VZV which were resistant to acyclovir (ACV), phosphonoacetic acid (PAA) or bromodeoxyuridine (BUDR) were also sensitive to IFN β, their average ID_{50} being 1.31 IU/ml. Analysis of virus-specific proteins by the immunofluorescent technique with various antisera showed that IFN had a significant effect before early protein synthesis.

INTRODUCTION

Varicella zoster virus (VZV) is a member of the herpes virus group, and can cause two distinct clinical conditions, chickenpox (varicella) and shingles (herpes zoster). The disease is usually mild and self-resolving, but the symptoms can be severe or even fatal in immunocompromised patients. Antiviral drugs such as adenosine arabinoside (Ara-A) and acyclovir (ACV) are used in treatment of herpes group viruses, but vary widely in their efficacy against VZV (Biron and Elion, 1980; Elion *et al,* 1977; Lerner and Bailey, 1976; May *et al,* 1977; Shigeta *et al,* 1983). Interferon (IFN) also has an antiviral effect against VZV (Arvin *et al,* 1978). For increasing the efficacy or reducing the toxicity, combinations of antiviral agents have been used (Ayisi *et al,* 1980; Fiala *et al,* 1974; Fischer *et al,* 1979; Wigand and Hassinger, 1980). ACV and interferon in combination show additive to synergistic effects against VZV (Baba *et al,* 1984). However, studies on the effects of interferon upon VZV have mainly been concerned with the effects of leukocyte interferon (IFN-α) (Arvin *et al,* 1978; Baba *et al,* 1984), and little is known about the effects of IFN-β and IFN-γ on VZV infection. Moreover the effects of IFN on drug-resistant VZV strains have not been tested. In this study, we examined the antiviral activity of human IFN-α, β and γ against VZV and that of IFN-β against drug resistant mutants of VZV by plaque reduction assay. We also compared the effects of combinations of IFN-β and IFN-γ, on VZV replication, with those of IFN-β and IFN-γ singly.

MATERIALS AND METHODS

Cells

Human embryonic fibroblast (HEF) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) as growth medium and 3% as maintenance medium. Cells were used for experiments at the 5th to 25th passage.

Viruses

The Kawaguchi strain was used as the parental strain of VZV (Yamanishi *et al,* 1980). Mutants with resistance to acyclovir (A1, A6 and A8 strains), Phosphonoacetic acid (PR strain) and bromodeoxyuridine (BR strain) were used as drug resistant viruses (Shiraki *et al,* 1983). Herpes simplex virus type 1 (seibert strain) was also prepared in HEF cells. Cell-free preparations of all VZV strains were obtained as described previously (Yamanishi *et al,* 1980), and were stored at -70°C until use.

Interferons

Human leukocyte IFN-α (1.1×10^7 IU/ml, 3×10^6 IU/mg), human fibroblast IFN-β (1.8×10^6 IU/ml, 2×10^7 IU/mg) and recombinant IFN-γ (2.5×10^6 IU/ml, 1×10^7 IU/mg) which was synthesized in *E. coli,* were supplied by Toray Industries Inc Tokyo. Interferon activity was assayed by measuring inhibition of the cytopathic effects of vesicular stomatitis virus (VSV) on fibroblast lung (FL) cells, and IFN titers are expressed in international reference units (IU).

Plaque reduction assay

The plaque reduction assay was used to determine the inhibitory effect of IFN on VZV replication. Confluent monolayers of HEF cells in 60 mm diameter tissue culture plates (Corning, USA) were pretreated with various concentrations of the IFNs singly or in combination for 24 hours. Then approximately 100 plaque forming units (PFU) of each VZV strain were inoculated onto the cultures. The plates were incubated for 1 hour at 37°C to allow viral adsorption, and then washed with phosphate buffered saline and incubated in maintenance medium for 4 days at 37°C. Then the cultures were fixed with 10% formalin, stained with 0.03% methylene blue, and plaques were counted under a dissecting microscope.

For studies on HSV, HEF cells pretreated with IFN were infected with approximately 100 PFU of HSV, then overlaid with maintenance medium containing 0.5% agarose. All tests were run in duplicate. The number of plaques was counted and the value for 50% reduction in plaque formation (ID_{50} = 50%-inhibitory dose) was calculated based on the numbers in control cultures infected with VZV or HSV.

Immunofluorescence studies

HEF cells were grown on 15 mm round glass cover slips resting in 24-flat-botton wells. Monolayers of both control and IFN-treated cells were prepared. Then 2 ml of maintenance medium containing IFN-β (10^5 IU/ml) were added and the coverslips were incubated at 37°C for 24 hours. The medium was removed by aspiration and the cells were infected with VZV (Kawaguchi strain). After an adsorption period of 1 hour at 37°C, the cultures were washed with phosphate buffered saline (PBS), flooded with maintenance medium and incubated at 37°C. For the immunofluorescence test, infected cells grown on cover slips were harvested at various times after infection (24 and 48 hours), washed with PBS, air dried and fixed in acetone at -20°C for 20 minutes. They were then stored at -20°C for staining.

For immunofluorescence staining, the fixed cultures on cover slips were each layered over with about 0.1 ml of antithymidine kinase antibody prepared in rabbits as described previously (Shiraki *et al,* 1985) and mouse monoclonal antibody, clone 8, which react with a glycoprotein of VZV (Okuno *et al,* 1983). After incubation at 37°C for 30 minutes in a humidified chamber, the cover slips were washed with PBS and layered over with anti-rabbit IgG or anti-mouse IgG conjugated with fluorescien isothiocyanate (Cappel, USA). After further incubation at 37°C for 30 minutes they were again washed with PBS, mounted in a 1 : 9 mixture of PBS and glycerin and observed by fluorescence microscopy.

RESULTS

Antiviral activities of IFN against VZV and HSV

Plaque reduction assays were used to determine the effects of IFN-α, -β and -γ on VZV (Kawaguchi strain), and results are shown in Fig 1. The inhibition of virus multiplication depended on the concentration and type of IFN. High concentrations of the IFNs completely inhibited viral multiplication. The ID_{50} values of IFN-α, -β and -γ were 0.813, 0.65 and 13.75 IU/ml, respectively. These

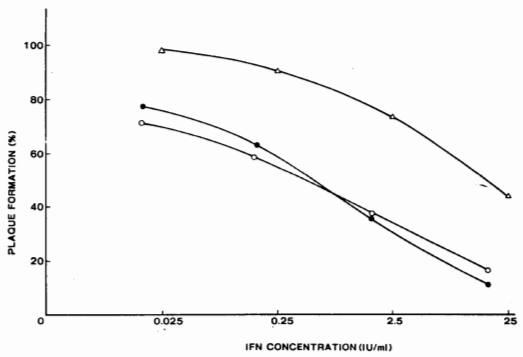

Fig 1—Inhibitions of VZV plaque formation by IFN-α, -β and -γ.
○IFN-α; ●IFN-β; △IFN-γ.

ID_{50} values showed that VZV was sensitive to all there IFNs and was most sensitive to IFN-β. The effects of these IFNs on HSV were tested in the same way, and ID_{50} values of 18.0, 10.38 and 115.0 IU/ml, respectively, were obtained (Fig 2). Thus although these IFNs all inhibited HSV replication, their ID_{50} values were higher for HSV than for VZV.

Effects of IFN-β on drug-resistant strains of VZV

The effects of IFN- β on various drug-resistant mutants of VZV were examined and the ID_{50} values obtained are shown in Table 1. The ID_{50} values for the various mutants differed, but all the mutants were sensitive to IFN. The average ID_{50} value of the drug-resistant strains was 1.31 IU/ml, while that of wild strain was 0.650 IU/ml. This suggests that the mechanism of the antiviral effect of IFN on VZV is different from that of antiherpes drugs.

Synergistic effects of IFNs

The effects of various concentrations of IFN- β and IFN- γ alone and in combination were examined. As shown in Table 2, plaque formation of VZV was inhibited 50 percent by 1.8 IU/ml of IFN-β , but for the same level of inhibition more than 25 IU/ml of IFN- α was required. The effects of all combinations of IFN- β and IFN- γ , assessed by the formula of Spector *et al* (1982), were additive to synergistic. Studied are required on the mechanism of the synergistic actions of IFNs, but this observation is encouraging for trial of combinations of IFNs against VZV infection.

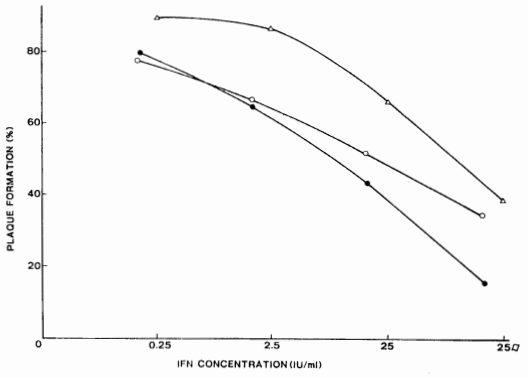

Fig 2—Inhibitions of HSV plaque formation by IFN-α, -β and -γ.
○IFN-α; ●IFN-β; △IFN-γ.

Table 1

Inhibition of various drug resistant strains of VZV by IFN.

Strain	Plaque reduction assay
	ID_{50} (IU/ml)
Parental strain	0.650
Drug resistant mutant; A1	0.828
A6	1.735
A8	0.763
PR	0.925
BR	2.286

HEF cells were treated with IFN-β for 24 hours and cultures were infected with VZV. Cultures were incubated for 4 days and stained as described in Materials and Methods.

Table 2

Effects of IFN B and alone and in combination on plaque formation by VZV.

IFN B	Plaque no. (% control) IFN			
(IU/ml)	0	2.5	25	250
0	100[a]	83	50	0
0.18	83	49 (69)[b]	25 (42)	0 (0)
1.8	42	26 (35)	11 (21)	0 (0)
18	4	4 (4)	0 (2)	0 (0)

a: Plaque reduction assays were performed in duplicate as described in the text.
b: Figures in parentheses show expected values calculated by the formula of Spector *et al* (1982).

Immunofluoresence test

We studied the mechanism of action of IFN by examining its effects on VZV 24 hours and 48 hours after infection, as early and late stages of infection. Effects were examined by the immunofluorescence technique with anti-thymidine kinase serum and a monoclonal antibody against a glycoprotein of VZV. HEF cells were cultured with

Fig 3—Fluorescence photomicrographs of virus-specific antigens in VZV-infected cultures. (a) (c) (e) IFN-treated and (b) (d) (f) un-treated; (a) (b) treated with McAb cl 8, specific for gpI; (c)-(f) treated with anti-TK rabbit serum; (a) (b) (c) (d) cells were fixed 24 hours after infection, (e) (f) cells were fixed 48 hours after infection.

IFN in maintenance medium for 24 hours before virus inoculation. As shown in Fig 3, 24 hours after infection virus-specific antigen was observed in only a few cells in infected, IFN-treated cultures, but in many cells in infected, untreated cultures. At 48 hours after infection, more antigen-positive cells had started to appear in IFN-treated cultures. These results showed that IFN acts early during viral replication before to early protein expression and consequently delays expression of viral antigen.

DISCUSSION

IFNs are known to have antiviral activity, and there are many reports on the *in vivo* and *in vitro* effects of IFNs in herpes viral infections. VZV belongs to the herpesvirus group and some of its biological characteristics seem to be similar to those of HSV. In this work we found that VZV was more sensitive than HSV to IFNs (Figs 1, 2). We examined the effects of IFNs on VZV replication *in vitro* and the results may reflect antiviral activity of IFN. We found by plaque reduction assay that the ID_{50} values of IFN α, β and γ against VZV were 0.813, 0.650 and 13.75 IU/ml, respectively. Thus IFN-γ and -β were highly effective against VZV, but IFN-γ was less effective. These results may reflect the findings that type 1 IFN (IFN α/β) and type II IFN (IFN γ) appear to lack homology and use different receptors at the cell surface (Branca and Baglioni, 1981). These differences might result in biological differences in the actions of these IFNs. Blalock *et al* (1980) reported that IFN α/β had more antiviral activity than IFN γ against VSV but less cellular activity than IFN γ in WISH and Hep-2 cells. Rubin and Gupta (1980) showed that the replication of VSV and encephalomyocarditis virus was inhibited more strongly by type I IFN whereas those of reovirus and vaccinia virus were more sensitive to type II IFN. The sensitivity of HSV to IFN depends on the target cells; HSV is highly sensitive to IFN when cultured in mouse macrophages (Domke *et al,* 1985). Thus the activity depends not only on the type of IFN but also on the types of virus and infected cells. In our study, IFNs had less effect on HSV than on VZV in HEF cells.

We found that IFN also inhibited the replication of various drug-resistant mutants of VZV, which are resistant to ACV, PAA or BUdR. These results indicate that IFN itself has a different mechanism of action from antiherpes drugs. Antiherpes drugs usually inhibit viral DNA replication by being incorporated into viral DNA or interfering with viral DNA polymerase (Elion *et al,* 1977), but interferon appears to inhibit replication of VSV at the level of transcription (Belkosi and Sen, 1987). In this work, immunofluorescence studies showed a difference between IFN-treated and control cells in the first 24 hours after infection: VZV was detected in only a few cell in IFN-treated cultures, but in many neighboring cells in the control cultures. This shows that IFN inhibits VZV in such a way that it can not complete a single life cycle and spread to other cells within 24 hours. In untreated cultures, a single cycle of virus growth requires 8 to 14 hours and after 18 hours progeny

virus start to spread to neighboring cells (Yamanishi *et al,* 1980).

In combination, IFNs have been found to have additive effects against herpesviruses (Czarniecki *et al,* 1984; Domke *et al,* 1985; Yamamoto *et al,* 1987). We also found that in combination IFN β and IFN β had additive to synergistic effects against VZV. Fleischmann *et al* (1979) observed that IFNB had more activity than IFN γ against mengo virus and that their combination resulted in great enhancement or potentiation of antiviral potection. These synergistic effects of IFNs suggest that individual IFNs have different mechanisms of action. These mechanisms of action are unknown, but our results are encouraging for use of IFNs, especially combinations of IFNs, in therapy of VZV infection and also for use of IFN against drug resistant strains of VZV.

In IFN-treated cells we observed inhibition of viral protein synthesis by immunofluorescence studies with antibody against thymidine kinase (TK), which is expressed in the early stage of viral replication, and with monoclonal antibody against a glycoprotein that is expressed in the late stage. In the case of HSV infection, some groups have reported that IFN inhibits a late stage in viral replication (Chatterjee *et al,* 1984, 1985; Munoz and Carrasco, 1984), and others have found that IFN inhibits an early stage (Domke *et al,* 1985, 1986; Gloger and Panet, 1984; Straub *et al,* 1986). We did not detect any immediate early protein by immunofluorescence, but our findings indicated that IFN blocks a step before early protein synthesis, since TK and this glycoprotein are early and late proteins, respectively.

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