Inhibition of *Herpesvirus hominis* Replication by Human Interferon

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The effect of human interferon on single-cycle replication of *Herpesvirus hominis* type 1 (HV-1) and vesicular stomatitis virus (VSV) in human foreskin fibroblast cultures (HFF) was studied. After treatment of HFF cultures with low concentrations (58 U) of human interferon, a variable but statistically significant inhibition of HV-1 was observed. At higher concentrations (greater than 95 U), yield reduction of HV in interferon-treated cultures approached that of VSV. Preliminary data indicate that antiviral activity decays more rapidly for HV-1 than for VSV after removal of interferon from cultures.

Members of the herpesvirus group are reported to be resistant to the antiviral activity of exogenous interferon in human cells when inhibition of cytopathic effect (CPE) is used to evaluate inhibition of virus replication (1, 3, 6). Others, however, have shown that herpesvirus CPE is inhibited by interferon inducers such as poly(I):poly(C) (8). Thus, the question of whether the herpesviruses are sensitive to the antiviral effects of interferon in a human cell system is not yet answered. In view of the increasing interest in the use of human interferon as an antiviral agent in human herpesvirus infections (5), quantitative data on the ability of human interferon to inhibit herpesvirus replication would be of value. Therefore, this study was done to evaluate the sensitivity of *Herpesvirus hominis* to inhibition by human interferon using quantitation of virus yields after single-cycle replication in interferon-treated human cell cultures.

**MATERIALS AND METHODS**

Viruses. *H. hominis* type 1 (HV-1, strain MacIntyre) and HV-2 (strain MS) were obtained from the American Type Culture Collection and grown in primary rabbit kidney cultures. Vesicular stomatitis virus (VSV, Indiana strain) was grown in human neonatal foreskin fibroblast (HFF) cultures. Newcastle disease virus (NDV) was obtained from allantoic fluids of 10- to 12-day-old embryonated hen eggs, harvested 2 days after virus inoculation.

Cell cultures. HFF cultures were prepared from foreskins treated with 0.25% (wt/vol) trypsin for 45 min. After centrifugation at 3,000 × g for 0.5 h, cells were resuspended in minimal essential medium (MEM) supplemented with 5% newborn calf serum (NBCS). Foreskins, trypsinized individually, yielded sufficient cells to prepare a single 60-mm petri dish culture. After outgrowth of the primary tissue, cells were dispersed with trypsin, and cultures were pooled and propagated as monolayers in 32-ounce (about 960 ml) glass screw-capped bottles. HFF cultures were subcultured weekly for 10 passages. MEM with 5% NBCS was used for both growth and maintenance of HFF cultures.

Primary rabbit kidney cultures were prepared from kidneys of 2- to 3-week-old New Zealand white rabbits. Cell suspensions were dispersed by three cycles of trypsin treatment over a 3-h period. The trypsinized cells were centrifuged at 600 × g for 10 min and then diluted 1:300 (vol/vol) for growth in MEM supplemented with 5% NBCS. Monolayer cultures were prepared in six-well multi-dish cultures (Linbro Chemical Co.; New Haven, Conn.) and used for virus assay 7 days after initial preparation.

The preparation of chick embryo fibroblast cultures has been previously described (7).

Preparation of interferon. Human interferon internal standard (NDV-IS) was prepared in this laboratory from HFF cultures inoculated with NDV at an input multiplicity of 0.1 plaque-forming unit (PFU) per cell. Supernatants from infected cultures were harvested 48 h after inoculation and adjusted to pH 2.0 with 1 N HCl. Five days later, supernatants were readjusted to pH 7.2 with 1 N NaOH and supplemented with 0.25% bovine serum albumin. Human interferon preparations so prepared were free of residual NDV PFU when assayed without dilution on chicken embryo fibroblast cultures, had no antiviral activity on chicken embryo fibroblast cultures against VSV, and required longer than 6 h to confer antiviral activity to HFF cells.

A sample of human interferon designated as FS-4 and titering 130,000 reference units per ml was generously supplied by Jan Vilcek, New York University Medical School.

**Interferon assays.** Interferon antiviral activity
was assayed both by reduction of PFU and reduction of virus yields during single-cycle replication. For determination of PFU reducing units of human interferon, monolayer cultures of HFF were grown in 6-well multi-dishes. Cultures were incubated overnight with 1 ml of sample diluted in serum-free MEM, challenged with approximately 50 PFU of VSV after removal of sample, and then overlaid with MEM containing agar (Difco) and 5% NBCS. Two days later monolayers were stained with a 1:10,000 dilution of neutral red in phosphate-buffered saline (PBS) and plaques were counted. Interferon plaque-reducing units were calculated graphically by probit analysis with 1 U defined as that dilution which reduced PFU by 50%.

Interferon assay by virus yield reduction was in HFF cells grown in tube cultures with approximately $10^5$ cells per tube. After incubation for 18 h with interferon diluted in serum-free MEM, cultures were challenged with 0.1 ml of virus at an input multiplicity of 10 PFU per cell. Control cultures were incubated in serum-free MEM before virus challenge. After virus adsorption for 2 h, cultures were washed twice with PBS and then reincubated for a period of one cycle of virus replication. Cultures were harvested by freezing at −70 C and then were rapidly thawed and refrozen for an additional cycle before assay for PFU on rabbit kidney cells. Interferon yield-reducing units were calculated graphically by probit analysis, with 1 U defined as that dilution which reduced virus yield by 50%.

The mean titer of the NDV-IS interferon was 132 ± 40 plaque-reducing units per ml based on 14 separate assays. In a single assay the VSV yield-reducing interferon titers of NDV-IS and FS-4 were compared to that of the reference interferon 69/19, which contains 5,000 U by definition, when assayed by plaque reduction against VSV. Yield reducing titers were 580, 76,000, and 8,800 U per ml, respectively. Similarly, plaque-reducing titers determined in the same assay were 230, 20,000, and 2,333 U per ml.

**RESULTS**

**Single-cycle replication of HV and VSV.**

HFF tube cultures were infected with HV-1, HV-2, and VSV at an input multiplicity of 10. After 2 h adsorption, cultures were washed with PBS incubated at 37 C in MEM with 1% NBCS, and harvested by freezing at 4-h intervals at the periods shown in Fig. 1. The period of single-cycle replication, as determined by PFU assay, was 12 h for VSV, 20 h for HV-1, and 16 h for HV-2 in HFF cultures.

**Inhibition of HV yields by human interferons.** Table 1 shows the effect of human

![Graph showing single-cycle replication of HV-1 (○), HV-2 (●), and VSV (△) in HFF cultures. HFF cultures were infected at an input multiplicity of 10 with the indicated viruses. After a 2-h adsorption period at 37 C, cultures were washed three times with PBS, replenished with MEM with 1% NBCS, and reincubated at 37 C for the indicated periods. Cultures were harvested for PFU assay by freezing at the time periods shown.](http://iai.asm.org/)

**Fig. 1. Single-cycle replication of HV-1 (○), HV-2 (●), and VSV (△) in HFF cultures. HFF cultures were infected at an input multiplicity of 10 with the indicated viruses. After a 2-h adsorption period at 37 C, cultures were washed three times with PBS, replenished with MEM with 1% NBCS, and reincubated at 37 C for the indicated periods. Cultures were harvested for PFU assay by freezing at the time periods shown.**

**Table 1. Effect of human interferon on single-cycle replication yields of HV-1 and VSV in HFF cell cultures**

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<th>Expt</th>
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<td>IF$^a$</td>
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$^a$ Cultures incubated for 18 h with 58 U of human interferon (IF) determined by VSV yield reduction assay.

$^b$ Cultures incubated for 18 h with serum-free MEM.

* $P < 0.0025$ for significance of difference between means of HV-1 yields in IF and control cultures by Student’s $t$ test analysis for paired samples.

* $P < 0.0005$ for significance of difference between means of VSV yields in IF and control cultures by Student’s $t$ test analysis for paired samples.
interferon NDV-IS on single-cycle replication of HV-1. VSV was included in all assays as a positive control for interferon antiviral activity. In a series of eight independent experiments, HFF cultures were incubated for 18 h with approximately 58 U of interferon and then challenged with virus. Replicate cultures were pooled as pairs, then assayed for PFU on rabbit kidney cultures. Variation in ability of interferon to inhibit virus replication was noted from experiment to experiment. However, in the series of eight experiments, the mean titer of HV-1 in interferon-treated cultures was significantly lower than controls \((P < 0.0025)\). Similarly, VSV yields were significantly lower \((P < 0.0005)\) in interferon-treated than in control cultures.

To determine whether the variation observed in the ability of interferon to inhibit virus replication was the result of intersubject variation, experiments were done in which eight replicate cultures were harvested, pooled as pairs, and assayed for PFU. The means ± 1 standard deviation in interferon-treated and control cultures infected with HV-1 were 4.9 ± 0.2 and 6.8 ± 0.1, respectively. Similarly, mean titers for VSV in interferon-treated and control cultures were 3.3 ± 0.2 and 8.0 ± 0.1.

Figure 2 shows the effect of a high titer interferon preparation, FS-4 interferon, on yields on HV-1, HV-2, and VSV. In cultures treated with 760 U of interferon, yields of both HV-1 and HV-2 were inhibited by greater than 2 logs. The inhibition of virus yield declined in a linear fashion with decreasing interferon concentration. The slopes of the lines representing yield inhibition of HV were similar to each other, but differed from the slope of the line representing yield inhibition of VSV replication. At similar dilutions of interferon, i.e., 1:8 or approximately 95 U, inhibition of VSV was approximately 0.5 log greater than HV-1.

**Kinetics of decay of interferon antiviral activity.** In a single experiment, replicate HFF cultures were incubated for 18 h with three concentrations of FS-4 interferon. After removal of interferon, cultures were either challenged with virus or replenished with media and reincubated for various intervals before virus challenge. Control cultures were challenged at each interval simultaneously with interferon-treated cells. The decay of interferon antiviral effects 1 day after removal of 12 U of interferon was similar for both HV-1 and VSV (Fig. 3). However, differences in the rate of loss were apparent in cultures treated with 58 or 760 U. Approximately 90% of antiviral activity for HV-1 was lost 1 day after removal of interferon. However, virus yields in cultures challenged with VSV 1 day after removal of either 58 or 760 U of interferon were not appreciably different from yields of cultures challenged immediately after overnight incubation with interferon.

Between 3 and 7 days antiviral activity rose in cultures exposed to 760 U of interferon and challenged with either VSV or HV-1. This increase in antiviral activity declined by 9 days after interferon removal. A similar increase in antiviral activity was detected between 5 and 7 days in cultures treated with 95 and 12 U of interferon and challenged with HV-1. This temporary period of increased antiviral activity was followed by a sharp decline in all cultures between 7 and 9 days after interferon treatment. Virus yields in replicate control cultures were essentially identical during the course of the experiment.

**Effect of human interferon on HV plaque formation.** HFF cultures were incubated overnight with approximately 100 U of human interferon NDV-IS and then used for PFU assay of HV-1, HV-2, and VSV. PFU counted 5 days after inoculation of cultures for HV and after 2 days for VSV were compared to those in controls. Reductions in PFU of 1.7, greater than 2.0, and greater than 3.3 logs were observed for HV-1, HV-2, and VSV, respectively.

**DISCUSSION**

The data presented in this study have shown that HV in a human cell system is subject to
inhibition by interferon during single-cycle virus replication. Previous studies have suggested that HV are resistant to the antiviral effects of human interferon when inhibition of CPE is used to evaluate inhibition of virus replication (1). However, CPE is not necessarily a correlate of virus replication. For example, Joklik and Merigan (4) have shown that interferon inhibits vaccinia virus deoxyribonucleic acid polymerase, virus deoxyribonucleic acid, and virus protein synthesis, yet does not protect from CPE. Thus, earlier conclusions based on observations of CPE may not reflect a true status with respect to inhibition of replication by interferon.

If one compares the effects of human interferon on inhibition of HV and VSV replication, HV is less sensitive to the inhibition by human interferon than VSV, particularly with low concentrations of interferon. With increasing concentrations of interferon, HV inhibition approached that achieved for VSV, within the limits of sensitivity of the assay system. The presence of residual VSV at high input multiplicity of infection prevents detection of yield inhibition greater than approximately 3 logs. Differences in interferon sensitivity of HV-1 and HV-2 were not observed with the virus strains used in this study.

Differences in susceptibility of HV and VSV to inhibition by interferon in yield reduction assays may be the result of a more rapid initial decay of antiviral activity for HV than for VSV, particularly after removal of high concentrations of interferon. One day after removal of greater than 12 U of interferon, antiviral activity for VSV is almost totally retained, but greater than 90% is lost for HV-1 (Fig. 3). A similar rapid decay of resistance has been reported for other viruses (2). Despite the initial rapid decay of interferon activity for HV-1, interferon-treated cultures were still effective in reducing HV PFU, counted 5 days after removal of interferon.

Preliminary data suggests that human interferon has different inhibitory effects for HV-1 than for VSV in human cells. First the relationship of virus inhibition to interferon concentration shows that slopes of dose-response lines are distinct for HV-1 and VSV. Second, decay of inhibitory activity against HV-1 appears to occur more rapidly than for VSV. These observations lend additional support to the suggestion of Youngner and his colleagues that separate resistance factors for ribonucleic acid and deoxyribonucleic acid viruses may develop in interferon-treated cell cultures. The biphasic decay of interferon activity for HV-1 shown in Fig. 3 was an unexpected observation and requires additional experimental confirmation.

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