Synthesis of Herpes Simplex Virus Proteins in Interferon-treated Human Cells

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SUMMARY

Pretreatment of HeLa cells with human interferon (IFN) resulted in the inhibition of herpes simplex virus (HSV) replication. We examined the stages in the replication of HSV type 1 and type 2 that were affected by IFN. The rate of synthesis of the HSV immediate-early (α) proteins was inhibited in IFN-pretreated HeLa cells. The subsequent inductions of HSV early (β) genes, determined by measuring the levels of cytoplasmic mRNA specific for the thymidine kinase, as well as the DNA polymerase enzyme activity, were also suppressed in the IFN-pretreated cells. These results indicate that IFN inhibits HSV replication primarily at a very early step, either prior to, or during the synthesis of α-proteins.

Herpes simplex virus (HSV) replication can be divided into three major stages according to the cascade order of virus protein synthesis. The synthesis of the three major HSV-1 immediate-early α proteins (ICP0, ICP4 and ICP27) is initiated at about 1 h after infection and these proteins appear to trigger the induction of the early β proteins (Honess & Roizman, 1975). The HSV-1-coded enzymes, thymidine kinase (TK) and DNA polymerase, which are induced a few hours after infection, have been identified as β proteins (Garfinke & McAuslan, 1974; Powell & Purifoy, 1977). The γ proteins, mostly virus structural proteins, are synthesized during the late stage of infection, concomitant with the synthesis of viral DNA. Blocking the expression of any set of early proteins results in subsequent inhibition of induction of the later virus genes in the cascade and finally in reduction of HSV yields (Marsden et al., 1976).

Several investigators have shown that HSV yields are reduced in interferon (IFN)-treated cells, but there is very little information as to the mechanism of inhibition (Rasmussen & Farley, 1975; Lerner & Bailey, 1976). Examination of the effects of IFN on the induction of the TK and DNA polymerase enzymes in mouse L-cells has shown that at relatively high concentrations, IFN suppressed the specific enzyme activities of these β proteins (Panet & Falk, 1983). In the present study, we describe the effects of human IFN on the induction and synthesis of the immediate-early (α), early (β) and late (γ) proteins after infection of human cells with HSV-1 and HSV-2.

Monolayer HeLa cells were grown in RPMI 1640 medium containing 10% calf serum. Stocks of the HSV-1, NIH strain, and the HSV-2, Curtis strain, were grown in BSC1 cells and titres were determined by plaque assay on BSC1 cells with an agar overlay. For virus infection, 2 × 10^5 HeLa cells were seeded in 35 mm plates. After 24 to 48 h, the medium was changed and cells were exposed to HuIFN-α for 18 to 24 h. The medium was removed and virus (0.3 ml) was added for 30 min at a m.o.i. of 5. After virus adsorption, the cultures were washed and fed with RPMI 1640 medium containing 10% calf serum (1 ml). Human leukocyte IFN (10^7 units/mg protein) (Cantell & Hirvonen, 1978), was obtained from T. Bino and H. Rosenberg of the Biological Institute, Nes-Ziona, Israel.

HeLa cells were selected for this study since the replication of RNA viruses (reovirus, encephalomyocarditis virus) is very sensitive to human IFN, and the IFN-induced enzymes have been extensively studied in these cells (Silverman et al., 1982; Nilsen et al., 1982).
HSV-1 infection of HeLa cells pretreated with HuIFN-α (2000 units/ml) resulted in a virus yield reduction of 1 to 2 log₁₀ as analysed by plaque assay on BSC1 indicator cells. To determine which stage of HSV replication is repressed by IFN, the rates of synthesis of the α, β and γ proteins were studied. The infected cells were pulse-labelled with [³⁵S]methionine 5 h after infection, and the proteins were resolved by gel electrophoresis (Fig. 1). In order to synchronize the synthesis of α proteins, cells were infected in the presence of cycloheximide (CHA) for 5 h and the proteins were pulse-labelled with [³⁵S]methionine for 1 h upon removal of the CHA (Pereira et al., 1977). The synthesis of the three major HSV-1 α proteins (ICP4, ICP0 and ICP27) was clearly inhibited in the IFN-treated cells (compare lanes 5 and 6). We identified the α proteins in infected cells by their synthesis immediately after removal of CHA and by comparing their molecular weights to the published values for HSV-1 proteins (Morse et al., 1978). The synthesis of HSV-1 β (ICP6 and ICP8) and some of the γ proteins (such as ICP25) in cells infected without CHA were also inhibited by IFN (compare lanes 3 and 4). Similar results were obtained when IFN-treated HeLa cells were infected with HSV-2. IFN inhibited the synthesis of HSV-2 α proteins (ICP4 and ICP27; compare lanes 9 and 10), as well as the synthesis of β and γ proteins (ICP6, ICP8 and ICP25), which are made at 6 h post-infection in the absence
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Fig. 2. Kinetics of HSV-1 DNA polymerase in IFN-treated (2000 units/ml) cells. HeLa cells were infected with HSV-1 and harvested at various times for the determination of HSV-1 DNA polymerase specific activity, as described by Panet & Falk (1983) and Purifoy & Benyesh-Melnick (1975). O, Control not treated with IFN; ●, treated with IFN during the infection period only (0 to 24 h); ■, treated with IFN for 18 h prior to infection; ⧫, treated with IFN for 18 h prior to infection and during the infection period.

Fig. 3. Effect of IFN on levels of TK mRNA in HSV-1-infected cells. HeLa cells (5 x 10^6) in 150 mm plates were treated with IFN (1500 units/ml) for 24 h, and infected with HSV-1 (m.o.i. 4) for 5 h. Total cytoplasmic RNA was purified by phenol extractions as described by Favaloro et al. (1980). Increasing amounts of RNA were spotted on a nitrocellulose filter and dot blot hybridization was carried out with nick-translated ^32P-pBRTK as a probe (BamHI fragment of HSV-1 DNA cloned in pBR322; Wigler et al., 1977). Hybridization was quantified by cutting the filters and counting in a scintillation counter. O, Control cells; ●, IFN-treated cells. Hybridization with similar amounts of RNA from mock-infected cells gave background radioactivity (20 c.p.m.). This value was subtracted from each point presented in the figure.

of CHA (compare lanes 7 and 8). In the gels presented in Fig. 1, the synthesis of HSV-1 proteins appeared somewhat more sensitive to IFN than that of the HSV-2 proteins. In repeated experiments, however, suppression of proteins encoded by the two viruses was not significantly different.

In cells infected without IFN, the synthesis of a major cellular protein (designated C) was partially inhibited as the infection proceeded due to HSV-induced shut-off (Fig. 1, lanes 3 and 7) (Read & Frenkel, 1983). However, IFN treatment, which clearly resulted in the suppression of viral protein synthesis, had no inhibitory effect on the synthesis of the cellular protein (C).

Since the expression of β genes in HSV-1 infection depends on the formation of α proteins, we compared the induction of DNA polymerase enzyme activity during a single-cycle infection of IFN-treated and control cultures (Fig. 2). From the enzyme assays, a more quantitative estimation of the effect of IFN on β gene induction could be derived. Induction of DNA polymerase was delayed in the IFN-pretreated cells; i.e. early after HSV-1 infection, the enzyme-specific activity was very low compared to the control cells. Thus, 3 h after infection, DNA polymerase activity was eightfold lower in the IFN-treated than in control cells. The inhibition appeared to decrease as infection progressed (10 to 24 h) since, whereas accumulation of DNA polymerase slowed down 5 h after infection of control cells, it proceeded linearly in the IFN-treated cells. For maximal inhibition, IFN had to be added 18 h prior to infection; addition of IFN only at the time of infection had no effect on the induction of DNA polymerase (Fig. 2).
To investigate whether IFN suppresses HSV-1 β mRNA, thus affecting the induction of β proteins, the steady-state levels of TK mRNA were measured 5 h after infection with HSV-1 (Fig. 3). Total cytoplasmic RNA was isolated from the infected cultures, control or pretreated with IFN, dotted on a nitrocellulose filter and hybridized with a $^{32}$P-labelled TK DNA probe. The filters were washed and the extent of hybridization was estimated by counting the radioactivity in the filters. The concentration of TK mRNA was 50% lower in the IFN-treated than in the control cell preparation (Fig. 3). Although the apparent inhibition of HSV proteins by IFN was stronger (Fig. 1 and 2), the twofold reduction in TK mRNA was significant and reproducible in four independent experiments.

We describe, in this work, the effects of IFN on the synthesis of α, β and γ proteins in HSV-1- and HSV-2-infected human cells. The results indicated that pretreatment with relatively high concentrations of HuIFN-α inhibited the rate of synthesis of the immediate-early α proteins, without affecting synthesis of cellular proteins. Since early functions are required for induction of the β and γ genes, the apparent suppression by IFN of β and γ protein translation could be, at least partially, a result of the earlier block. Thus, expression of β genes appears to be affected at both the transcription and translation levels in the IFN-treated cells. The inhibition of β protein (DNA polymerase) accumulation in IFN-treated cells is largely of a transient nature. This could be because even at high concentrations of IFN there is a residual synthesis of α proteins which may suffice for the slow induction of β genes but at a later stage of infection.

It should be noted that the effect of IFN on HSV, as well as on many other viruses, depends on the multiplicity of virus infection (Panet & Falk, 1983). In the present work, a relatively high multiplicity of HSV infection had to be used in order to detect the synthesis of viral proteins. Under these conditions, IFN delayed the cytopathic effect of HSV-1 20 h after infection and inhibited virus yields in HeLa cells by 10- to 100-fold (data not shown). Different multiplicities of infection, among other factors, may explain the conflicting reports as to the relative sensitivities of HSV types 1 and 2 to IFN (Overall et al., 1980; Lerner & Bailey, 1976). In our experimental system, the two HSV strains exhibited similar sensitivities to HuIFN-α. Relatively high concentrations of IFN were needed for HSV inhibition. Comparison of HuIFN-α and HuIFN-β dose responses indicated that concentrations higher than 1000 units/ml of the two preparations were required for significant virus inhibition (data not shown).

IFN has been shown to inhibit various viruses at different levels of replication (for review, see Lengyel, 1982). For example, simian virus 40 is primarily inhibited at a very early step, that leading to the transcription of T-antigen mRNA, while many of the RNA viruses are inhibited at the translation of viral proteins. Retroviruses appear to be inhibited during budding of virions through the cell membrane, and penetration through the cell membrane by infecting vesicular stomatis virus was also inhibited by IFN (Whitaker-Dowling et al., 1983). The present study indicates that herpes simplex viruses are primarily inhibited at the translation of the α gene products. More work, however, is required to establish whether IFN also affects the transcription of the immediate-early genes (α) and, perhaps, penetration of the infecting HSV.

The inhibition by IFN of another member of the herpesvirus group has also been studied in some detail. The synthesis of an immediate-early protein of human cytomegalovirus was inhibited after infection of IFN-treated cells (Stinski et al., 1982). Whether all members of the herpesvirus group are similarly inhibited at an early step after infection, at the synthesis of the immediate-early proteins, awaits further comparative studies.

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REFERENCES


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