Inhibition of Transcription of Herpes Simplex Virus Immediate Early Genes in Interferon-treated Human Cells

By FROMA OBERMAN AND AMOS PANET*

Department of Virology, The Hebrew University Hadassah Medical School, Jerusalem, Israel

(Accepted 12 February 1988)

SUMMARY

The effect of interferon (IFN) treatment on the early stages of herpes simplex virus type 1 (HSV-1) replication in three types of human cells was investigated. Interferon pretreatment was shown to reduce the steady state levels of both total and polysome-bound HSV-1 immediate early α mRNAs. Using the nuclear run-off transcription assay, we showed that IFN selectively inhibited transcription of the HSV-1 genes, with no effect on transcription of total cellular RNA or that of the β-tubulin RNA. Thus, IFN appears to inhibit the initiation of HSV-1 α gene transcription rather than affect the stability of the respective mRNAs. IFN did not prevent the HSV-1-induced early shut-off of host cellular protein synthesis caused by a structural protein of the infecting virus. This observation indicated that the IFN-mediated inhibition of HSV-1 replication is at a stage beyond viral penetration into the cytoplasm. These results suggested that IFN blocked HSV-1 replication primarily at a very early stage, during the onset of α mRNA transcription.

INTRODUCTION

Interferon (IFN) inhibits different viruses at various stages of virus replication. For many RNA viruses, growth appears to be blocked primarily at the level of mRNA translation. Retroviruses, on the other hand, are inhibited at the level of virus assembly and release (Lengyel, 1982). Of the DNA viruses, the effect of IFN on simian virus 40 (SV40) replication has been studied extensively and the onset of early transcription appears to be markedly inhibited (Brennan & Stark, 1983).

The inhibition by IFN of HSV-1 growth has been demonstrated in several studies, but the stage at which IFN inhibits the replication cycle remains unclear. Muñoz & Carrasco (1984) reported no major inhibition of HSV-1 protein synthesis in IFN-treated cells, and suggested that the progeny virus produced was non-infectious. The results of Chatterjee et al. (1984, 1985) indicated that IFN acted by preventing the release of virion particles from IFN-treated cells, implying that IFN acted late in the viral replication cycle, after protein synthesis had been completed. In contrast, it has been demonstrated (Gloger & Panet, 1984) that treatment of HeLa cells with human IFN inhibited HSV-1 replication either prior to, or during, the synthesis of the immediate early α proteins. Reports by Domke et al. (1983, 1986) and Straub et al. (1986) have also indicated that pretreatment of both mouse and human macrophages with low doses of IFN efficiently inhibited viral replication primarily at the level of HSV-1 immediate early α protein synthesis. The discrepancy among the various reports might be due to the different cell types or different IFN preparations used.

In the present study we investigated the effects of recombinant human IFN-α during the early stages of HSV-1 replication, on the transcription of the immediate early α genes, in several types of human cells. By applying a nuclear run-off transcription assay, we demonstrated that pretreatment with IFN prevented HSV-1 replication, acting primarily at the initiation of transcription.

0000-8160 © 1988 SGM
Methods

Cell cultures and viruses. HEp-2 and HEp-2 human cell lines were grown at 37 °C in RPMI medium containing 5% foetal calf serum (FCS). SH human diploid fibroblasts were obtained at passage eight and grown in Dulbecco's minimal essential medium (DMEM) containing 10% FCS. Stocks of HSV-1 (NIH strain) were prepared in HEp-2 cells and titres determined by plaque assay on BSC-1 cells (Panet & Falk, 1983). Encephalomyocarditis (EMC) virus was grown and titrated in mouse L cells (Panet & Falk, 1983). Human leukocyte IFN-α was obtained from Hoffman La Roche (N.J., U.S.A.) and titrated with vesicular stomatitis virus (VSV) on HEp-2 cells. Cell cultures were treated with IFN for 24 h in DMEM containing 10% FCS. The medium was then removed and, after washing of the cells with additional medium, they were infected with HSV-1 as indicated in the figure legends.

HSV-1 DNA polymerase activity. Cell extracts were prepared and enzyme activities determined as described by Panet & Falk (1983) and Purifoy & Benyesh-Melnick (1975). In order to suppress cellular DNA polymerases 0.1 M-(NH₄)₂SO₄ was included in the assay mixture. The incorporation of [3H]TTP into activated DNA template—primer was determined after precipitation with TCA.

Analysis of viral proteins. Infected cell cultures were pulse-labelled for 1 h with [35S]methionine (50 μCi/ml) in methionine-free MEM, lysed in SDS sample buffer and electrophoresed in SDS–polyacrylamide gels (12.5%), as described by Gloger & Panet (1984). Gels were treated for fluorography, dried and exposed to X-ray film at −70 °C.

Isolation of RNA. Total RNA was isolated from infected cells by homogenization of the cells in guanidinium isothiocyanate, followed by precipitation in 4 M-lithium chloride and phenol/chloroform extraction (Cathala et al., 1983). RNA was quantified by spectrophotometry at 260 nm.

Polysomes were prepared from the cytosol using the MgCl₂ precipitation method of Palmiter (1974). This involved homogenizing the cells in a cold lysis buffer (125 mM-Tris–HCl pH 7.5, 25 mM-NaCl, 5 mM-MgCl₂, 200 μg/ml heparin, 0.5% Triton X-100), and centrifuging the homogenate for 5 min at 3000 g, and 5 min at 15 000 g. MgCl₂ was added to a final concentration of 120 mM, and the extract was incubated on ice for 75 min. The sample was then layered onto a cushion of 1 M-sucrose in the above buffer without Triton X-100, and the polysomes were pelleted by centrifugation at 27 000 g for 10 min. mRNA and rRNA were extracted from polysomes by phenol extractions (Maniatis et al., 1982).

For each experiment cells were scraped in phosphate-buffered saline with 20% glycerol and, prior to RNA extraction, part of the cell suspension was set aside to assay for DNA polymerase induction, to ensure that the IFN was effective.

RNA electrophoresis and Northern blot analysis. Total cytoplasmic and polysomal RNAs were electrophoresed on 1:1% agarose gels containing 1.2% formaldehyde, transferred to nitrocellulose, and hybridized as described (Maniatis et al., 1982). Size markers (28S and 18S rRNA) were visualized by staining the nitrocellulose filters with methylene blue after hybridizations were completed.

DNA probes. The DNA plasmids used to probe for HSV-1 α genes were pSG1-EK1, pSG1-ES1 (Quinlan & Knipe, 1985) and pRB115 (Mackem & Roizman, 1980). pSG1-EK1 and pSG1-ES1, provided by David Knipe (Harvard Medical School, Boston, Mass., U.S.A.), had been derived from the pSG1 plasmid which contains the EcoRI J–K fragment of HSV-1 [map coordinates 0 to 0.08, 0.83 to 0.865 (0.965 to 1.0); Quinlan & Knipe, 1985]. pSG1-EK1 which primarily codes for the ICP-4 gene, but also overlaps with the ICP-0 gene, had been constructed by cloning the EcoRI–KpnI restriction fragment of pSG1 in pMM102. pSG1-ES1, which codes for the entire ICP-0 gene as well as a β-gene which encodes an mRNA of about 4.3 kb (Harris-Hamilton & Bachenheimer, 1985), had been constructed by cloning the EcoR1–SacI restriction fragment of pSG1 in pUC18. The pRB115 plasmid, which contains the HSV-1 BamHI S-P fragment [map coordinates 0 to 0.022, 0.80 to 0.844, 0.975 to 1.0] cloned into pBR322, codes for both the ICP-4 and ICP-0 genes. pBRTK (Wigler et al., 1977) which contains an HSV-1 BamHI fragment [map coordinates 0.290 to 0.312] cloned into pBR322 codes for the thymidine kinase (TK) gene. pBR-tubulin, a BamHI fragment of human β-tubulin cDNA cloned into pBR322, and pMuLV-LTR (Honigman & Panet, 1983) a Moloney murine leukaemia proviral DNA cloned in pBR322 were also used. The appropriate fragments were isolated and labelled with [32P]dCTP by nick translation (Rigby et al., 1977).

Measurements of run-off transcription. HEp-2 cells were infected at an m.o.i. of 10. Procedures for the isolation of nuclei and the run-off transcription assay were performed as described (Greenberg & Ziff, 1984; Godowski & Knipe, 1986) except that the hybridization was for 48 h at 55 °C in a solution of 50% formamide, 5 × SSC, 50 mM-phosphate buffer pH 7.0, 50 μg/ml sonicated salmon sperm DNA, 1 × Denhardt's solution (0.02% w/v of each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) and 0.1% SDS. Conditions of high stringency were necessary to avoid non-specific hybridization between the 32P-labelled RNA of uninfected cells and the HSV-1 DNA plasmids. We typically isolated 5 × 10⁶ c.p.m. 32P-labelled RNA per 10⁶ cells, and found that IFN did not affect the amount of TCA-precipitable radioactive RNA per cell. Equal amounts of TCA-precipitable 32P-labelled RNA were added to hybridization reaction in a given experiment. After hybridization, the filters were washed four times in a buffer containing 5 × SSC, 0.1% SDS, 1 mM-EDTA and 10 mM-Tris–HCl pH 7.5, at 55 °C, for 30 min each rinse. Filters were then incubated with 10 μg/ml RNase A in 0.3 M-NaCl for 10 min at 37 °C and rinsed with 0.3 M-NaCl at 25 °C (Marzluff & Huang, 1984).
Inhibition of HSV-1 by interferon

To prepare filters for hybridization with $^{32}$P-labelled RNA from run-off transcription experiments, plasmid DNAs were linearized by digestion with restriction enzymes, denatured with 0·2 M-NaOH for 30 min and neutralized with 6 x SSC (Greenberg & Ziff, 1984). DNA samples were spotted onto nitrocellulose filters (5 μg/slot) using a slot blot apparatus.

**Determination of virus-induced cellular protein shut-off.** HeLa cells (50000) and SH diploid fibroblasts (20000), seeded in 24-well dishes, were infected with HSV-1 at increasing multiplicities in the presence of actinomycin D (2·5 μg/ml). At 3 to 4 h post infection (p.i.), the cells were pulse-labelled with $[^3H]$leucine (4 μCi/ml) in leucine-free MEM, and the incorporation of $[^3H]$leucine into TCA-insoluble material was determined (Panet, 1983).

**RESULTS**

Inhibition of HSV-1 DNA polymerase by IFN

Several studies using a variety of cell lines have indicated that the replication of HSV-1 is inhibited by IFN, as determined by the plaque reduction assay (Cayley et al., 1984; Domke et al., 1985; Gloger & Panet, 1984; Panet & Falk, 1983). Although the different cells varied in their sensitivity to IFN, the level of HSV-1 inhibition was 10- to 100-fold. As shown previously for HSV-1-infected mouse L cells, IFN suppressed the synthesis of the early β proteins, TK and DNA polymerase (Panet & Falk, 1983). Furthermore, in HeLa cells, the induction of both HSV-1 immediate early α and early β proteins was inhibited by pretreatment of the cells with IFN (Gloger & Panet, 1984).

High concentrations of IFN were required, however, to suppress significantly the protein synthesis of HSV-1 as compared to other virus groups. This made study of the mechanisms responsible for HSV-1 inhibition by IFN relatively difficult. It was therefore of interest to determine whether there exist other human cells more sensitive to IFN's effect on HSV-1. Several established human cell lines, as well as diploid cell cultures of low passage number, were pretreated with increasing concentrations of IFN, and infected with HSV-1. Five h after infection, the cells were harvested, extracts were prepared, and HSV-1 DNA polymerase activity was assayed. We found that different cell cultures exhibited varying levels of sensitivity to the IFN-mediated inhibition of DNA polymerase induction. From the range of cultures tested, the SH diploid fibroblasts (Fig. 1 a) and HEp-2 cell line (Fig. 1 b) were found to be significantly more sensitive to IFN than HeLa cells upon virus infection. Whereas IFN, at the maximum dose of 3200 units/ml caused less than 40% inhibition of HSV-1 DNA polymerase induction in HeLa cells, 50% inhibition was attained at 400 units/ml and 250 units/ml for HEp-2 and SH diploid fibroblast cells respectively. It should be noted that HSV-1 replicates efficiently in all three cell types. However, higher multiplicities of HSV-1 were necessary for the infection of SH cells, as compared to HeLa and HEp-2 cells, to reach the same degree of virus replication, as indicated by DNA polymerase induction. This phenomenon may be attributed either to the fact that the HSV-1 used in these experiments had been propagated in HeLa cells or to an inherited difference of cell sensitivities to HSV-1.

**Effect of IFN on the infection of SH diploid fibroblasts by EMC virus and HSV-1**

Previous studies have shown that for most of the standard cell lines used in IFN research HSV-1 replication exhibited a much lower sensitivity to IFN relative to the RNA viruses such as EMC virus (Cayley et al., 1984; Panet & Falk, 1983). Since the replication of HSV-1 seems to be very sensitive to IFN in SH diploid fibroblasts, we compared the effect of IFN on HSV-1 and EMC virus protein synthesis in these cells (Fig. 2).

For the SH diploid fibroblasts, the same concentration of IFN provided a similar degree of inhibition of both HSV-1 and EMC virus proteins. As illustrated in Fig. 2, a relatively low concentration of IFN (100 units/ml) is sufficient to inhibit significantly the synthesis of the EMC viral proteins e, F and γ (compare lanes 1 and 2), and the HSV-1 proteins ICP-6, ICP-8, ICP-10 and ICP-25 (compare lanes 4 and 5). IFN at 800 units/ml caused an even stronger inhibition, but again a comparable reduction of protein synthesis was observed for both of these viruses (lanes 3 and 6). It should be noted that many HSV-1 proteins are induced 6 h p.i., but only four major proteins were characterized (Fig. 3).

Cellular protein synthesis, as indicated by the major cellular protein (C) of $M_\text{f}$ 45000 was only slightly affected after EMC virus infection (compare lanes M and 1), in accordance with
Fig. 1. Dose response of IFN for the inhibition of HSV-1 DNA polymerase in three human cell lines. 200000 HeLa cells (●), 200000 HEp-2 cells (○) and 100000 SH diploid fibroblast cells (▼) were plated in 35 mm dishes and after 24 h treated with increasing concentrations of IFN for an additional 24 h. (a) HeLa and HEp-2 cells were infected at an m.o.i. of 5. (b) HeLa cells were infected at an m.o.i. of 5 and SH diploid fibroblasts at an m.o.i. of 50. After 5 h of infection, cells were harvested and HSV-1 DNA polymerase activities were assayed (Gloger & Panet, 1984; Purifoy & Benyesh-Melnick, 1975). The protein content of cell extracts were determined according to Lowry et al. (1951) and were found to be unaffected by the IFN pretreatment. Results are given as a percentage of DNA polymerase activity of control cultures not treated with IFN. DNA polymerase activity in the control cell extracts (100%) represented for HeLa and HEp-2 incorporation of 80000 and 36000 c.p.m. respectively (a) and for HeLa and SH diploid fibroblasts incorporation of 40000 and 24000 c.p.m. respectively (b).

Fig. 2. Effect of IFN on EMC virus and HSV-1 protein synthesis. SH diploid fibroblasts (100000 cells) were plated in 60 mm dishes and after 24 h were either left untreated (lanes M, 1 and 4), or treated for an additional 24 h with IFN at 100 units/ml (lanes 2 and 5) and 800 units/ml (lanes 3 and 6). Cells were infected with either EMC virus at an m.o.i. of 10 (lanes 1 to 3) or HSV-1 at an m.o.i. of 50 (lanes 4 to 6). At 5 to 6 h p.i. cultures were pulse-labelled with [35S]methionine, harvested, and the extracts subjected to gel electrophoresis (Methods). Equal amounts of radioactive material were loaded in each slot.

previous observations (Jen et al., 1980). Thus, pretreatment with IFN selectively inhibited viral protein synthesis (lanes 2 and 3). Infection with HSV-1 resulted in a partial shut-off of cellular protein synthesis (Read & Frenkel, 1983), and consequently the selectivity of IFN action is less pronounced. (Compare protein C in lanes M, 4, 5 and 6.)

In similar experiments conducted with HEp-2 cells, comparable results were obtained (data not presented). With this cell line, IFN at 200 units/ml was sufficient to inhibit strongly both EMC and HSV-1 viral protein synthesis.

Effect of IFN on HSV-1 α mRNAs

Previous work had indicated that IFN blocked the translation of α proteins ICP-4 and ICP-0 (Gloger & Panet, 1984). To determine what effect IFN has on the steady state levels of HSV-1 α
Inhibition of HSV-1 by interferon

Fig. 3. Effect of IFN on the levels of α mRNA in HSV-1-infected SH diploid fibroblasts. SH diploid fibroblasts (500000 cells) were plated in 150 mm dishes and after 48 h the medium was changed. Control cultures (lane 1), or cultures pretreated with IFN (200 units/ml) (lane 2), were infected with HSV-1 at an m.o.i. of 50. Total cytoplasmic RNA was isolated at 3 h p.i. and equal amounts of RNA (20 µg) were subjected to gel electrophoresis. Northern blot analysis was carried out and probed for ICP-0 and ICP-4 mRNAs with pSG1-ES1 (a), and pSG1-EK1 (b) as described in Methods. 18S and 28S rRNA standards are indicated. Coordinates of the probes in relation to the α mRNAs are illustrated in (c). EcoRI sites are represented as E, KpnI as K and SacI as S.

mRNAs, cytoplasmic RNA was isolated at 3 h p.i. from IFN-treated and control SH diploid fibroblast cells. Northern blot analysis was performed on equal amounts of RNA using the pSG1-ES1 (Fig. 3a) and pSG1-EK1 (Fig. 3b) DNA probes, which hybridize primarily to the 2.7 kb ICP-0 and 4.7 kb ICP-4 mRNAs respectively. The overlap between pSG1-EK1 and the DNA coding region of ICP-0 results in some hybridization between this probe and the 2.7 kb ICP-0 mRNA (Quinlan & Knipe, 1985). In addition to the ICP-0 mRNA, pSG1-ES1 hybridizes to an unidentified β mRNA of 4.3 kb which, like that of ICP-0, maps within the IRL region of the HSV-1 genome (Harris-Hamilton & Bachenheimer, 1985). Accumulation of this β mRNA was detected at this time. Pretreatment of the SH diploid fibroblasts with only 200 units/ml IFN significantly decreased the steady state levels of both the ICP-0 (Fig. 3a, compare lanes 1 and 2) and ICP-4 mRNAs (Fig. 3b, compare lanes 1 and 2).

We next investigated the possibility that the reduction in the steady state levels of HSV-1 α mRNA in IFN-treated cells was due to a preferential inhibition in the binding of viral mRNA to polysomes, thereby reducing mRNA stability. As polysome fractions could not be prepared reproducibly from SH diploid fibroblasts, we performed these experiments with infected HeLa cells. Northern blot hybridizations using the pSG1-ES1 probe (Fig. 4a) and the pSG1-EK1 probe (Fig. 4b) were done on total mRNA fractions (lanes 1 and 2) as well as on polysome-bound mRNA (lanes 3 and 4). It is evident that IFN inhibited the polysome-bound ICP-4 and ICP-0 mRNAs to the same extent as it did the total steady state mRNA levels. As the ratio of the total to polysomal mRNA remained constant for IFN-treated and control cells, it appears that IFN does not affect the distribution between free and polysome-bound α mRNAs in the cytoplasm.
Effect of IFN on α mRNA transcription rates

The reduction in steady state levels of α mRNAs may be a result either of the effect of IFN on the rate of transcription or on the stability of the mRNAs. To investigate the effect of IFN on the initiation of α mRNA transcription more directly, we employed the HSV-1 nuclear run-off assay (Godowski & Knipe, 1986; Weinheimer & McKnight, 1987). For this in vitro system, nuclei were isolated from infected cells and incubated with nucleoside triphosphates and [α-32P]UTP. This reaction permits the engaged RNA polymerase molecules to elongate nascent transcripts. The radioactive material was purified and equal amounts of TCA-precipitable radioactive material from each RNA sample were hybridized to HSV-1 DNA fragments that had been bound to nitrocellulose filters. The amount of radiolabelled RNA hybridized with each viral probe is a measure of the number of RNA polymerase II molecules that were transcribing each gene at the time the nuclei were isolated. Therefore, this assay provides a direct measure of the effect of IFN on the rate of transcription.

Because the SH diploid fibroblasts did not produce efficient transcription with this assay, we have developed a run-off transcription assay with HEp-2 cells. Nuclei isolated from IFN-treated HEp-2 cells 6 h p.i. showed a striking decrease in transcription of the three HSV-1 genes tested, ICP-0, ICP-4 and TK (Fig. 5), but total synthesis of RNA was not affected. To analyse whether the effect of IFN was specific for HSV-1 gene transcription, we used a probe for a cellular gene, β-tubulin. Over-exposure of the autoradiogram was necessary to visualize hybridization to the human β-tubulin gene clearly. IFN had no effect on the transcription of the β-tubulin gene in the same nuclei, indicating that it is involved in preferentially blocking viral gene transcription. It is possible that the run-off transcription of α genes observed at middle and late times of the infectious cycle does not accurately reflect authentic transcription (Godowski & Knipe, 1986; Weinheimer & McKnight, 1987). We therefore repeated the run-off transcription assay at 3 h.
Inhibition of HSV-1 by interferon

Fig. 5. Effect of IFN on the transcriptional activity of HSV-1 α genes. HEp-2 cells (10^6) were plated in 150 mm dishes and after 48 h medium was changed. Control cultures (−), or cultures pretreated with IFN (500 units/ml) (+), were infected with HSV-1 (m.o.i. 10) for 6 h and their nuclei were isolated. 32P-labelled RNA transcripts were synthesized in the isolated nuclei and hybridized to plasmid DNAs that had been spotted on nitrocellulose filters. The plasmids used were pRB115, pBRTK and pBR-tubulin which code for the HSV-1 ICP-4, ICP-0 TK and cellular β-tubulin genes respectively.

Fig. 6. Effect of IFN on the kinetics of HSV-1 gene induction. At 3 and 6 h after HSV-1 infection (m.o.i. 10), nuclei were isolated from HEp-2 cells, non-treated (−), or pretreated with IFN (500 units/ml) (+), as described in the legend to Fig. 5. 32P-labelled RNA transcripts were synthesized in the isolated nuclei and hybridized to plasmid DNAs that had been spotted onto nitrocellulose filters. The plasmids used were pSG1-EK1 and pSG1-ES1 which code for ICP-4 and ICP-0, pBRTK, pBR-tubulin and pMuLV-LTR.

Two inconsistencies were observed in the run-off transcription experiments 6 h p.i.; firstly, gene expression was high at 6 p.i. although synthesis of the corresponding α proteins is known to decrease during this period, and secondly, TK gene transcription at 6 h appears lower than that of the α genes. Both phenomena have been previously described for the HSV-1 run-off transcription system (Weinheimer & McKnight, 1987).

Effect of IFN on the HSV-1-induced shut-off of host protein synthesis

It was of interest to determine whether, in addition to its effect on α mRNA transcription, IFN inhibits at an even earlier stage of HSV-1 replication. It has been demonstrated that in cells infected with HSV-1, a structural protein of the infecting virions (vhs) causes the rapid
cessation of cellular protein synthesis (Read & Frenkel, 1983; Schek & Bachenheimer, 1985). To determine if this early shut-off event, which reflects virion penetration, occurs in IFN-treated cells, monolayers of HeLa and SH diploid fibroblast cells were infected with HSV-1 at increasing multiplicities. Actinomycin D was added upon infection to block all further transcriptional activity. At 3 to 4 h p.i. cells were pulse-labelled with [3H]leucine and the incorporation of [3H]leucine into TCA-insoluble material was determined. Results are given as percentages of the incorporation by non-infected cells. One-hundred per cent [3H]leucine incorporated represented for (a) SH diploid fibroblasts, control cultures 32000 c.p.m. and IFN pretreatment 25000 c.p.m. and for (b) HeLa cells, control cultures 45000 c.p.m. and IFN pretreatment 43000 c.p.m.

Fig. 7. Effect of IFN on the HSV-1-induced shut-off of cellular protein synthesis. (a) SH diploid fibroblasts, control (○), or pretreated with IFN (200 units/ml) (●) and (b) HeLa cells, control (○), or pretreated with IFN (2000 units/ml) (●) were infected with HSV-1 at increasing m.o.i. in the presence of actinomycin D (2.5 µg/ml). At 3 to 4 h p.i. the cells were pulse-labelled with [3H]leucine, and the incorporation of [3H]leucine into TCA-insoluble material was determined. Results are given as percentages of the incorporation by non-infected cells. One-hundred per cent [3H]leucine incorporated represented for (a) SH diploid fibroblasts, control cultures 32000 c.p.m. and IFN pretreatment 25000 c.p.m. and for (b) HeLa cells, control cultures 45000 c.p.m. and IFN pretreatment 43000 c.p.m.

These results indicate that IFN does not prevent the HSV-1-induced shut-off of host cell protein synthesis, and therefore suggest that the IFN-mediated inhibition of HSV-1 replication is at a stage beyond virion penetration.

DISCUSSION

Using the nuclear run-off transcription assay, we have demonstrated in the present work that IFN inhibits the onset of transcription of HSV-1 immediate early α gene. The inhibition appears to be specific, as total incorporation of [32P]UMP into RNA was not affected in the nuclei of IFN-pretreated cells. Moreover, although both the cellular gene β-tubulin and the HSV-1 α genes are transcribed by the same cellular RNA polymerase II (Spear & Roizman, 1980), inhibition was specific to the viral genes. This finding indicates that the reduced steady state levels of α mRNAs in IFN-treated cells is due to a block in synthesis rather than enhanced degradation of viral mRNA. As polysome-bound and total steady state α mRNAs were reduced...
Inhibition of HSV-1 by interferon

1175

to the same extent in the IFN-treated cells, it appeared that the binding of mRNA to ribosomes for the initiation of protein synthesis was not inhibited.

All of these data suggest, therefore, that the inhibition of synthesis of α proteins observed in IFN-treated cells (Domke et al., 1986; Gloger & Panet, 1984; Straub et al., 1986) is a consequence of an earlier inhibition at the transcription level. This early block is likely to affect all subsequent classes, β and γ mRNAs, whose transcription is dependent upon functional α proteins (Honess & Roizman, 1975). These observations do not, however, preclude the possibility that, in addition to the early block, IFN independently suppresses later stages of HSV-1 replication, thus augmenting the final inhibition of virus yield.

It is of interest to compare the effects of IFN on HSV-1 to that on SV40. Infection of IFN-pretreated monkey cells with SV40 resulted in the inhibition of viral early mRNA production, which affected all subsequent steps of virus replication (Brennan & Stark, 1983). Although not shown directly, it has been suggested that the onset of SV40 early gene transcription from partially uncoated SV40 virions is specifically inhibited in cells pretreated with IFN. Application of the run-off transcription assay to the SV40 system should resolve whether, like HSV-1, initiation of transcription is inhibited. Recently, Belkowski & Sen (1987) have provided evidence that the replication of VSV, a negative strand RNA virus, is also inhibited by IFN at the level of primary mRNA synthesis. This transcription is catalysed, however, by the RNA-dependent RNA polymerase introduced by the infecting virions. The common feature of infection by the three viruses HSV-1, SV40 and VSV is that primary transcription precedes translation as the first step of replication. The nature of the cellular repressors, induced in the interferon-treated cells to bring about specific inhibition of the onset of early transcription of these viruses, is not known. It is possible that uncoating of HSV-1 virions is inhibited in the IFN-treated cells, and therefore access for RNA polymerase II to the viral genome is limited. This proposed mode of IFN action would also explain the selective inhibition of HSV-1 α gene transcription in the infected cell.

Another stage which remains to be investigated is the effect of IFN on Vmw65, a virion-associated βγ polypeptide which specifically trans-activates α gene expression by stimulating α gene promoters (Campbell et al., 1984; O'Hare & Hayward, 1984). It is possible that the IFN-induced reduction of α mRNA transcription may be a direct consequence of the interaction between the IFN-induced system and the Vmw65 viral protein.

Previous studies were contradictory with respect to the pattern of HSV-1 inhibition by IFN (Chatterjee et al., 1985; Domke et al., 1986; Gloger & Panet, 1984; Straub et al., 1986). The different cells used in these studies varied in their sensitivity to IFN, and to HSV-1 infection, two parameters which might have affected the results. In an attempt to reconcile these data, we have compared the effect of IFN on the replication of HSV-1 in three human cell lines. Although the cells, HeLa, HEP-2 and SH diploid fibroblasts, showed comparable responses to the anti-EMC virus activity of IFN, they varied in their sensitivity to the anti-HSV-1 activity. Nevertheless, pretreatment with IFN resulted in the inhibition of α mRNA accumulation and α gene induction in the three lines. These results suggest that, despite the variation in their sensitivities to IFN, the mechanism of IFN action against HSV-1 is identical for the different cells.

We thank H. Falk for excellent technical assistance and Drs H. Jacobsen, H. Kirchner and S. Mittnacht for helpful discussions. This research was supported by the DKFZ, Heidelberg, Germany, The Israeli Council for Research and Development, Der Hessische Minister für Wissenschaft und Kunst, Germany, and Dr Karl and Dr Lea Terner Fund for Cancer Research.

REFERENCES


(Received 16 November 1987)