Summary

Reports on the arrest of herpes simplex virus type 1 (HSV-1) replication by interferon (IFN) are inconsistent. By the use of immunofluorescence and immunoblot assays with monoclonal and polyclonal antibodies, effective arrest of viral translation by human IFN-α in human fibroblasts was detected for the HSV-1 strains KOS and McIntyre. In HeLa cells which are less sensitive to IFN inhibition and in 444 cells, a HeLa-fibroblast hybrid cell line, the inhibition was less pronounced. These results confirm earlier observations that IFN or polyinosinic-polycytidylic acid block the replication of HSV-1 in human, monkey and mouse cells no later than the immediate early phase of infection.

Conclusions from experiments on the effects of interferon (IFN) on herpes simplex virus (HSV) genome expression in cell culture are only partially compatible with each other. In this paper, a review of pertinent investigations is given followed by the results of a reinvestigation of the effect of IFN on HSV type 1 (HSV-1)-specific translation.

Inhibition of early steps of HSV replication in cell culture by IFN inducers or IFN itself has been shown in several investigations. With polyinosinic-polycytidylic acid [poly(I)-poly(C)], an inducer of IFN-β, and primary African green monkey kidney cells, Brandner & Mueller (1973) and Lipp & Brandner (1980) documented a nearly complete impairment of HSV-1-specific protein and DNA synthesis. Later investigations were expanded by the use of diploid human fibroblasts and immortalized mouse cells and human leukocyte IFN-α and mouse IFN (from Newcastle disease virus-stimulated L cells), respectively. In all cell species, all classes of IFN used exerted a concentration-dependent arrest of the synthesis of all classes (α, β and γ) of HSV-1 proteins (Lipp, 1981; Lipp & Brandner, 1985). For example, 20 units of human leukocyte IFN-α per ml were sufficient to reduce the number of HSV-1 protein-positive human fibroblasts by more than 66%. However, in mouse cells bearing an integrated HSV-1 thymidine kinase gene, only the expression of superinfected virus was IFN-sensitive, whereas the translation of the integrated viral genome was IFN-resistant (Lipp, 1981; Lipp & Brandner, 1985). Panet & Falk (1983) and Lewis et al. (1983) have made the same observation.

In addition, a human leukocyte IFN-α-dependent decrease of normal length HSV-1-specific RNA was demonstrated by Northern blot analysis (Lipp, 1981; Lipp & Brandner, 1985). With poly(I)-poly(C) as an inducer of IFN-β, and human fibroblasts, the amounts of normal length RNA and the numbers of HSV-positive cells were reduced by more than 99%.

Thus, HSV-1-specific translation has been shown to be highly sensitive to natural IFN-α and -β in human, monkey and mouse cells. A parallel decrease in the pool of normal length HSV-1 transcripts also occurred in human diploid fibroblasts.

Later, Panet et al. (1985) presented a reinvestigation of the IFN effect on HSV-1 translation in cell culture. A carcinoma cell line with significantly lower IFN sensitivity (HeLa) and the NIH
strain of HSV-1 were used. Human leukocyte IFN-α and human fibroblast IFN-β inhibited all classes of HSV-1 proteins in that cell–virus combination. No impairment of HSV-1 transcription was found. Recently, Domke-Opitz et al. (1986) reconfirmed the IFN sensitivity of HSV-1 translation by using macrophages and natural and recombinant human IFN-α and -β.

The above investigations demonstrate the sensitivity of HSV-1 translation towards natural or recombinant IFN-α and -β of human, monkey or mouse origin. Muñoz & Carrasco (1984) published results not compatible with any of the foregoing. Their investigations were performed with the KOS strain of HSV-1, HeLa cells and human IFN-α from leukocytes. Though infectious virus progeny was reduced after pretreatment of cells with 1000 units of IFN-α per ml, there was little effect on the synthesis of any class of viral proteins, and even formation of virus particles was IFN-resistant. It was concluded that IFN leaves herpesvirus transcription, translation, genome replication and even morphogenesis intact and affects only the infectivity of the newly formed virus particles.

Chatterjee et al. (1985) presented a further reinvestigation using human fibroblasts, recombinant IFN-α and -β and the HSV-1 strains F and MP. As in the foregoing study no significant inhibition of virus-specific protein synthesis was found, but the formation of two virus capsid glycoproteins was reduced and virion morphogenesis and release were arrested.

The conclusions from the latter two investigations are clearly inconsistent with the main conclusions drawn from all of the other references above. Considering the possibility that the use of different strains of HSV-1 might be responsible for the apparently different IFN inhibition mechanisms, we began a renewed study. The strain KOS (Muñoz & Carrasco, 1984) caused the greatest differences from the results of our investigations which were carried out exclusively using the McIntyre strain (Lipp & Brandner, 1985). We therefore directly compared the McIntyre strain with KOS. In addition to low passage human fibroblasts (line α1), HeLa cells (Muñoz & Carrasco, 1984) and 444 cells, a non-oncogenic hybrid between HeLa and human fibroblasts, were used as hosts. Virus was propagated on Vero cells and titrated on α1 cells. All cell cultures were either free of mycoplasma contamination, ascertained by DNA fluorescence staining with Hoechst dye 33258, or were, in cases of slight contamination, decontaminated by treatment with 80 μg of ofloxacin per ml for one or two passages.

Human recombinant IFN-α2C (Hu. rIFN-α2C, from E. Boehringer, Vienna, Austria) was added in Eagle’s MEM (EMEM) with 3 to 5% foetal calf serum (FCS) 18 h prior to infection. Dual positive cell controls were performed in the absence and the presence of either cytosine arabinoside (40 μg/ml) or phosphonoacetate (100 μg/ml) during infection to prevent any HSV-1 DNA replication. Virus-specific protein synthesis was estimated by immunofluorescence staining (Fig. 1) and counting the HSV-1-positive cells 7 h after infection. The antibodies used were either human polyclonal or mouse monoclonal. The human antibodies were selected from patients with high anti-HSV-1 α and β protein serum titres (Lipp & Brandner, 1980, 1985). The monoclonal mouse antibodies were specific for the HSV-1 α protein ICP4 (Showalter et al., 1981). This analysis was paralleled by an immunoblot analysis of extracts from treated infected cells and controls. The immunofluorescence assays with the selected human polyclonal sera and the monoclonal mouse anti-ICP4 antibody revealed similar numbers of infected cells in all experiments.

In α1 cells both HSV-1 KOS and HSV-1 McIntyre protein synthesis turned out to be highly sensitive to inhibition by IFN (Fig. 2) and the IFN dose dependence was the same as published previously for the McIntyre strain (Lipp, 1981; Lipp & Brandner, 1985). Inhibitors of HSV-1 DNA synthesis had no influence on the number of positive cells or the inhibition by IFN (data not shown). This demonstrated that in untreated infected cell cultures no secondary cycles of viral DNA replication had occurred 7 h after infection. On the other hand, both HSV-1 KOS- and McIntyre-specific translation was less IFN-sensitive in HeLa than in α1 cells, but not totally resistant as observed by Muñoz and Carrasco (1984). The hybrid cell line 444 had an impaired IFN sensitivity similar to the parental HeLa cell line. Obviously fusion of HeLa cells with IFN-sensitive fibroblasts was insufficient to confer full IFN sensitivity.

Immunoblot analysis of cell extracts from IFN-treated infected cells exhibited inhibition of
Fig. 1. Immunofluorescence assay of ICP4 in IFN-α-treated HSV-1-infected ω1 and HeLa cells. Confluent cells grown in EMEM with 5% FCS on Lab-Tek cell culture chamber glass slides (Miles, no. 4808) were treated with 300 units/ml of Hu. rIFN-α2C for 18 h before infection with HSV-1 KOS (m.o.i. 4). After 7-8 h the cultures were rinsed with phosphate-buffered saline (PBS) and then fixed in methanol-acetone (1:1; −20 °C) for 10 min. Purified mouse monoclonal anti-ICP4 antibody diluted in PBS-0.1% NP40 was added to the cells for 1 h. After washing for 20 min, the slides were incubated with fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse IgG (Nordic Immunological Laboratories, code RAM/Ig/FITC), washed again and mounted with Citifluor to prevent fading. Photographs were taken with a Zeiss photomicroscope equipped with an epifluorescence condenser. (a) Pretreated with 300 units/ml of Hu. rIFN-α2C, infected; (b) untreated, infected; (c) untreated, uninfected.
HSV-1 translation in complete agreement with the quantitative immunofluorescence data (Fig. 3).

In summary, these observations allow us to conclude (i) that virus-specific protein synthesis is IFN-sensitive both in HSV-1 KOS- and McIntyre-infected cells, (ii) that this inhibition is more pronounced in human diploid cells than in transformed human cells and (iii) that this effect seems to be independent of the IFN class (α or β), species (human, monkey, murine) or source (natural or recombinant). The low IFN sensitivity of HeLa cells is well known (Lipp, 1981; Sakuragi & Simon, 1986).

This reinvestigation provides no clues to explain the variant findings of Muñoz & Carrasco (1984) and of Chatterjee et al. (1985). The question whether a particular step in HSV-1 genome expression, even before translation, could be arrested by IFN was not the goal of this reinvestigation, although from our previous studies with human fibroblasts the formation of normal viral transcripts is impaired (Lipp, 1981; Lipp & Brandner, 1985). Even this more detailed insight leaves the mechanism of the virus specificity of this process obscure. The synergistic action of tumour necrosis factor α plus IFN-γ causes an even more drastic inhibition
Fig. 3. Immunoblot assay of HSV-1 KOS proteins from α1 and HeLa cells. Cells were pretreated for 18 h with Hu.rIFN-αC2 and then infected with HSV-1 KOS, m.o.i. 3-5. After 1 h, the medium was changed. Seven h later, the cells were washed with cold PBS and lysed with a buffer containing 60 mM-Tris–HCl pH 6.8, 3% SDS, 10% glycerol and 5% mercaptoethanol. The lysate was centrifuged (10,000 g, 10 min) and aliquots were separated on a 10% SDS–polyacrylamide gel. The gel was fixed with 25% 2-propanol, 10% acetic acid and 65% H2O. Proteins were electroblotted onto Schleicher & Schuell cellulose nitrate paper (blotting buffer: 40 mM-glycine, 4% methanol, 5 mM-Tris–HCl pH 8.3; 30 V/cm; 18 h). The blots were incubated with 10% casein in PBS, washed in PBS–0.1% Tween 20 and incubated for 18 h in human anti-HSV-1 serum (1:200 in PBS–Tween). After washing, the blots were incubated for 1 h with peroxidase-labelled rabbit anti-human IgG (1:1000; Sigma). The label was visualized by incubation with 0.06% diaminobenzidine–HCl and 0.015% H2O2 in H2O for 15 min and photographed. (a) α1 cells; (b) HeLa cells. Lanes 1, uninfected controls; lanes 2 to 6, cells were treated with 0, 0, 10, 300 or 1000 units Hu.rIFN-αC2 per ml respectively. The positions of M₀ standards are indicated.

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REFERENCES


Short communication


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