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Antiviral Research 32 (1996) 27–34

Antiviral Research

Differential effect of recombinant human and mouse interferons on replication of herpes simplex virus type 1 in mouse cells

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Received 22 February 1996; accepted 29 April 1996

Abstract

Pretreatment of murine (BALB/3T3) cells with either murine or recombinant hybrid human B/D interferon (IFN) blocked the release of infectious herpes simplex virus type 1 (HSV-1) from treated cells. The block in replication was not due to an effect on attachment of HSV-1 to the target cells or to toxic effects of IFN. Immunoblot analyses showed that murine IFN significantly reduced the expression of virus-specific proteins in IFN-treated cells. In contrast, B/D IFN had no major effect on the expression of viral proteins in treated cells. In support of the above observation, electron microscopy of virus-infected cells displayed formation of nucleocapsids within the nucleus of IFN-treated cells. However, the expression of glycoproteins B and D was reduced in B/D IFN-treated cells. These results suggested that murine IFN blocked HSV-1 replication at an early stage whereas B/D IFN inhibited HSV-1 replication at a late stage in virus morphogenesis.

Keywords: Interferon; Interferon, mouse; Interferon, human, recombinant; Herpes simplex virus type 1

1. Introduction

Herpes simplex virus (HSV) causes a broad spectrum of illness ranging from asymptomatic infection to life-threatening disease (e.g. neonatal infection or encephalitis). In addition to acute infection, these viruses become latent in sensory ganglia and can reactivate, sometimes causing serious illness. Several antiviral drugs, especially nucleoside analogs and foscarnet, have been shown to be useful in the treatment of some of these diseases; however, the emergence of virus isolates which are resistant to existing antiviral

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PII S0166-3542(95)00975-9

agents and the known toxicity of some of these drugs have prompted continued research for new therapies for HSV diseases.

We and others have reported that human α and β IFNs blocked significantly the replication of HSV type 1 (HSV-1) in a variety of cells including human fibroblast and neuroblastoma cells (Munoz and Carrasco, 1984; Gloger and Panet, 1984; Chatterjee et al., 1985; Mittnacht et al., 1988; Chatterjee and Whitley, 1989; DeStasio and Taylor, 1990; Chatterjee and Burns, 1990; Maheshwari et al., 1994). Studies from different laboratories revealed that recombinant hybrid human IFNs, such as α B/D or α A/D, are also effective in preventing virus replication in heterologous cells such as murine fibroblast or monkey kidney cells (Meister et al., 1986; Horisberger and De Staritzky, 1987; Chatterjee and Whitley, 1989).

The exact mechanism of action of these IFNs against HSV replication is unknown. Several papers have reported conflicting results regarding the mechanism(s) of action of IFNs against HSV-1 replication. It has been reported that IFNs inhibited HSV replication at an early stage in the virus replication cycle (Gloger and Panet, 1984; Oberman and Panet, 1988; Mittnacht et al., 1988; DeStasio and Taylor, 1990). However, other studies suggested that IFNs blocked HSV replication at a late stage in virus morphogenesis (Munoz and Carrasco, 1984; Chatterjee et al., 1985; Chatterjee and Whitley, 1989; Chatterjee and Burns, 1990; Popik and Pitha, 1991). In this report, we demonstrate that both murine and human α B/D IFNs inhibited the release of infectious HSV-1 from treated murine 3T3 cells. However, murine and human α B/D IFNs affected differentially the expression of HSV-1 proteins in 3T3 cells. Our results suggested that the mode of action primarily depends on the type of IFN employed to block the virus replication.

2. Materials and methods

2.1. Cell cultures and virus

Mouse embryo fibroblast (BALB/3T3) and African green monkey kidney (BS-C-1) cells were obtained from the American Type Culture Collection (Rockville, MD). BALB/3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and gentamicin (50 μg/ml). BS-C-1 cells were grown in medium 199 containing 7.5% heat-inactivated FBS and gentamicin (50 μg/ml). The strain E-377 of HSV-1 was used throughout this study.

2.2. Reagents and radioisotopes

Murine IFN ($\alpha + \beta$) was purchased from Lee Biomolecular Research Inc. (San Diego, CA). Hu-IFN-α B/D was provided by Dr. Heinz-Kurt Hochkeppel (Ciba-Geigy, Basel, Switzerland). Reagents for polyacrylamide gel electrophoresis (PAGE) were obtained from Bio-Rad Laboratories (Richmond, CA). Na ^{125}I (15.5 mCi/μg of iodine) was obtained from Amersham Corp. (Arlington Heights, IL). Protein A was purchased from Pharmacia Laboratories (Uppsala, Sweden). Monoclonal antibodies against gB and gD were prepared in this laboratory (Koga et al., 1986). Human serum with HSV antibodies was obtained from M. Carr (California Pacific Medical Center, San Francisco, CA). Rabbit anti-mouse IgG was obtained from Organon Teknika Corp. (West Chester, PA).

2.3. Polyacrylamide gel electrophoresis and immunoblotting

Untreated and IFN-treated cell lysates were subjected to sodium dodecyl sulfate (SDS)-PAGE (9.5%) after infection with HSV-1 (Chatterjee et al., 1982). The fractionated proteins were then electrophoretically transferred to nitrocellulose paper as described previously (Towbin et al., 1979; Johnson et al., 1984; Chatterjee et al., 1985). Nitrocellulose strips were incubated at room temperature either with murine monoclonal antibodies against gB and gD or human serum containing HSV antibodies. The strips were further incubated with rabbit anti-mouse IgG for 30 min when murine monoclonal antibodies were used. Finally, the strips were treated with [^{125}I] protein A for 1 h at room temperature and processed for autoradio-

graphy. Protein A iodination was performed as described by Greenwood et al. (1963).

2.4. Electron microscopy

IFN-treated and untreated 3T3 cells, were processed for electron microscopy after HSV-1 infection, as previously described (Chatterjee et al., 1982). In brief, 3T3 cells grown in 60-mm dishes were treated with different concentrations of IFN. One set of cells served as untreated control. Cells were then infected with strain E-377 of HSV-1 as before. Samples were then washed with phosphate-buffered saline (PBS) 24 h postinfection and fixed with 1% glutaraldehyde. Cells were then post-fixed with 1% osmium tetroxide and embedded in an epoxy resin mixture. Thin sections were stained with uranyl acetate and lead citrate and were examined under a Philips EM 301 electron microscope.

3. Results

3.1. Effect of mouse and Hu-IFN-α B/D IFNs on the release of infectious HSV-1 from BALB/3T3 cells

In brief, cells were pretreated with different concentrations of murine and human α B/D IFNs for 18 h. Cells were then infected with E-377 strain of HSV-1 (multiplicity of infection (m.o.i.) = 1). One set of cells served as untreated control. Supernatant fluids were collected 24 h postinfection and tested for their ability to form plaques on BS-C-1 cells. The results of this experiment demonstrated that both IFNs blocked the release of infectious HSV-1 from treated 3T3 cells (Table 1). However, murine IFN was more effective against HSV-1 replication than human α B/D IFN in 3T3 cells. The 50% effective concentration (EC_{50}) for murine and α B/D IFNs was approximately 25 and 85 U/ml respectively.

In parallel, the effect of Hu-α B/D IFN on the release of infectious HSV-1 from human fibroblast cells was also determined. The result (Table 2) indicated that Hu-α B/D IFN was more effective against HSV-1 replication in human cells than in 3T3 cells.

Table 1
Effect of IFNs on the release of infectious HSV-1 from 3T3 cells

IFN	U/ml	PFU/ml	% Inhibition
None	–	3.5×10^5	0
Hu-α B/D	25	2.8×10^5	20.0
	50	2.2×10^5	37.1
	125	1.2×10^5	65.7
	250	1.0×10^5	71.4
	500	6.6×10^4	81.1
	1000	3.1×10^4	91.1
Murine IFN	10	2.3×10^5	34.3
	25	1.7×10^5	51.4
	50	9.8×10^4	72.0
	125	5.3×10^4	84.9
	250	2.3×10^4	93.4
	500	1.2×10^4	96.6

PFU, plaque-forming units

The block in release of virus particles was not due to toxic effects of these IFNs on the growth of 3T3 cells. No significant changes were observed after IFN treatment in cell number or in cell morphology. In brief, 3T3 cells were exposed to different concentrations of IFNs and the number of viable cells counted 72 h post-treatment. The result of this experiment (Table 3) showed that the inhibitory concentration (IC_{50}) for cell proliferation for both of these IFNs was greater than 10 000 U/ml. Thus, the selectivity index (SI) was > 400 for mouse IFN and > 100 for α B/D IFN. The selectivity index was determined by dividing IC_{50} by EC_{50}.

Table 2
Effect of Hu-α B/D IFN on the release of infectious HSV-1 from human fibroblast cells

U/ml	PFU/ml	% Inhibition
None	1.2×10^6	0
10	6.0×10^5	50.0
50	3.1×10^5	74.2
100	2.1×10^5	82.5
200	5.0×10^4	95.8

PFU, plaque-forming units

Table 3
Effect of IFNs on the growth of 3T3 cells

IFNs	U/ml	No. of cells post-treatment	
		0 h	72 h
None	–	4.4×10^5	2.4×10^6
Hu-α B/D	500		2.0×10^6
	1500		2.6×10^6
	5000		2.3×10^6
	10 000		2.9×10^6
Murine IFN	500		2.3×10^6
	1500		2.3×10^6
	5000		2.1×10^6
	10 000		2.1×10^6

3.2. Effect of murine and human α B/D IFNs on the adsorption of HSV-1 to 3T3 cells

To determine whether the inhibition in replication was due to the effect of these IFNs on the attachment of HSV-1 to target cells, 3T3 cells were pretreated with different concentrations of IFNs for 3 h at 37°C. One set of cells without any treatment served as an untreated control. Both treated and untreated cells were washed and infected with HSV-1. Supernatant fluids were collected after 90 min adsorption at room temperature and tested for the presence of unadsorbed virus by a plaque assay. As shown in Table 4, these IFNs had no effect on the attachment of HSV-1 to 3T3 cells.

Table 4
Effect of IFNs on the adsorption of HSV-1 to 3T3 cells

IFNs	U/ml	Residual infectivity (PFU/ml) [a]
None	–	6.2×10^3
Hu-α B/D	500	5.0×10^3
	1000	6.1×10^3
None	–	5.8×10^3
Murine IFN	500	6.4×10^3
	1000	6.9×10^3

PFU, plaque-forming units
[a] Virus added = 5.0×10^4/ml.

3.3. Expression of viral polypeptides and assembly of nucleocapsids in IFN-treated cells

In brief, 3T3 cells were pretreated with murine and human α B/D IFNs and then infected with HSV-1 as described before. Cell lysates were collected 24 h postinfection and processed for PAGE and immunoblotting. The resulting nitrocellulose blots were incubated with human anti-HSV-1 antibodies. The expression of HSV-1 proteins was significantly inhibited in murine IFN-treated 3T3 cells (Fig. 1A). However, no major effect on the expression of HSV-1 proteins was observed in α B/D IFN-treated 3T3 cells (Fig. 1B). Thus, a separate but identical blot resulting from α B/D IFN-treated cell lysate was incubated with monoclonal antibodies to gB and gD to examine whether the block in production of infectious particles was due to a defect in the expression of viral glycoproteins. It has been found that α B/D IFN significantly inhibited the expression of gB and gD in treated 3T3 cells (Fig. 1C). As expected, the expression of gB and gD was also inhibited in mouse IFN-treated 3T3 cells (data not shown). We also assessed the amounts of glycoproteins released from the infected cells treated with α B/D IFN. In brief, 3T3 cells were pretreated and infected with HSV-1 as before. Supernatant fluids were collected and the virus was pelleted by ultracentrifugation at 35 000 rpm for 1 h at 4°C in a Sorvall TH 641 rotor. The virus pellets were lysed and subjected to PAGE and subsequent immunoblotting. The nitrocellulose blot was reacted with monoclonal antibodies to gB and gD. The result of this experiment showed that α B/D IFN-treated cells released reduced amounts of gB and gD (Fig. 1D).

Consistent with the above finding, electron microscopy indicated the presence of nucleocapsids inside the nuclei of both untreated and α B/D IFN-treated cells (Fig. 2). Furthermore, several extracellular virus particles could also be observed in α B/D IFN-treated 3T3 cells (Fig. 2B). In contrast, few nucelocapsids were observed inside the nuclei of mouse IFN-treated cells (data not shown).

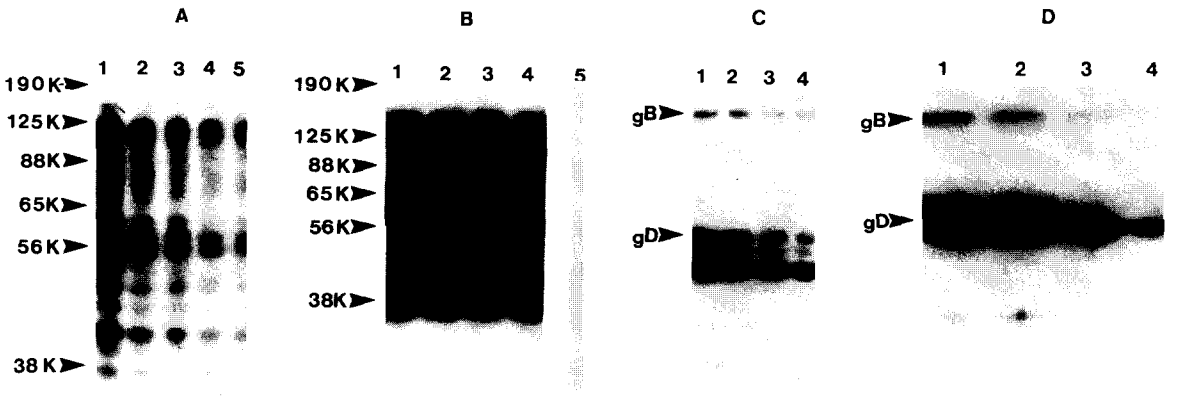

Fig. 1. Expression of viral proteins in IFN-treated 3T3 cells. (A) mouse IFN-treated cells; lane 1, no IFN; lane 2, 250 U/ml of IFN; lane 3, 500 U/ml; lane 4, 750 U/ml; lane 5, 1000 U/ml. (B) B/D IFN-treated cells; lane 1, no IFN; lane 2, 250 U/ml; lane 3, 500 U/ml; lane 4, 1000 U/ml; lane 5, uninfected control. (C) B/D IFN-treated cells; lane 1, no IFN; lane 2, 250 U/ml; lane 3, 500 U/ml; lane 4, 1000 U/ml; (D) expression of extracellular viral glycoproteins; lane 1, no IFN; lane 2, 250 U/ml; lane 3, 500 U/ml; lane 4, 1000 U/ml. (A) and (B), nitrocellulose blots were incubated with human HSV antibody-positive serum; (C) and (D), nitrocellulose blot was reacted with monoclonal antibodies to gB and gD.

4. Discussion

In this report, we demonstrated that both murine and Hu-α B/D IFNs inhibited the release of infectious HSV-1 from IFN-treated 3T3 cells. The block in replication was neither due to the effect of IFNs on the attachment of HSV-1 to the target cells nor due to toxic effect(s) of IFNs on these cells.

The exact mechanism of IFN action on HSV replication is poorly understood. Several papers reporting contradictory results have been published to describe the mode of action of IFNs against HSV replication. Reports from some laboratories indicated that human IFNs blocked HSV replication at an early stage prior to the expression of immediate-early (IE) genes (Gloger and Panet, 1984; Oberman and Panet, 1988; Mittnacht et al., 1988; DeStasio and Taylor, 1990). However, other laboratories suggested that IFNs did not block the early events in the HSV replication cycle (Munoz and Carrasco, 1984; Chatterjee et al., 1985; Chatterjee and Whitley, 1989; Chatterjee and Burns, 1990; Popik and Pitha, 1991). Munoz and Carrasco (1984) even demonstrated formation of extracellular herpesvirus particles (although non-infectious) in cells treated with human IFNs. Furthermore, it has recently been reported by Torigoe et al. (1993) that human IFNs inhibited the replication of cytomegalovirus, another member of the herpesvirus family at stages beyond the production of late proteins which supports our earlier observation (Chatterjee et al., 1985). The observed differences in the mode of action of human IFNs against herpesviruses was attributed to the use of different preparations and/or concentrations of IFNs, or use of different cell lines or different virus strains. Until now, however, experiments to resolve these possibilities have not been carried out. In this study, employing the same virus strain, the same cell line and the same concentrations of IFN, we demonstrated that the mode of action (i.e. inhibition at early or late stage of replication) predominantly depends on the type of IFNs used in the experiment. In 3T3 cells, murine IFN significantly reduced the expression of virus-specific proteins. In contrast, α B/D IFN had no major effect on the expression of nucleocapsid proteins in treated cells even at the highest concentration tested. In support of this observation, electron microscopy of virus-infected cells showed formation of distinct nucleocapsids within the nucleus of IFN-treated cells. However, the expression of gB and gD was significantly reduced in α B/D IFN-treated 3T3 cells. This differential effect of human IFN was also noticed

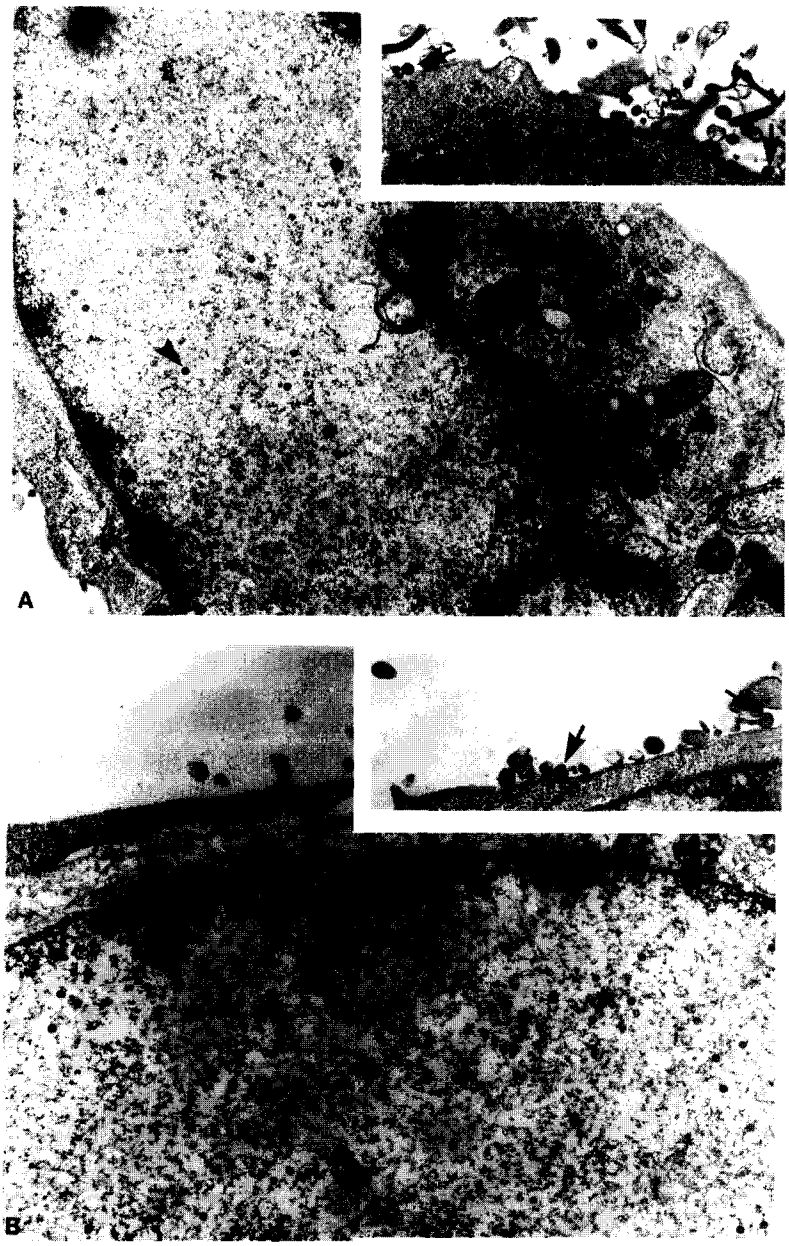

Fig. 2. Electron micrographs of HSV-1-infected and B/D IFN-treated, HSV-1-infected 3T3 cells. (A) untreated, HSV-1-infected cell. Note the distinct nucleocapsids inside the nucleus (arrowheads; 37 nucleocapsids per nucleus, mean) and extracellular virus particles (arrows). (B) IFN-treated, HSV-1-infected cell. Distinct nucleocapsids (arrowheads; 34 nucleocapsids per nucleus, mean) and extracellular virus particles (arrows) can be observed. Magnification: (A), (B), × 12 100.

when human α IFN or human recombinant α A/D IFN was used to block HSV-1 replication in human neuroblastoma or monkey kidney cells (Chatterjee and Whitley, 1989; Chatterjee and Burns, 1990). In addition, the synthesis of vesicular stomatitis virus G protein was also inhibited in

transfected COS cells treated with IFN (Sahni and Samuel, 1986). Although the exact mode of action of IFNs on the expression of HSV-1 glycoprotein is unknown, it is apparently different from that observed after treatment of target cells with tunicamycin or brefeldin A (Chatterjee et al., 1990; Chatterjee and Sarkar, 1992).

At present, the exact mode of action of murine IFN on HSV replication is not clear. It is possible that murine IFN affects a unique step in the complicated transcription-translation cascade that controls HSV-1 gene expression. Thus, future studies with murine IFN should help us to determine its exact mechanism of action against HSV replication in 3T3 cells.

Recent studies from this laboratory revealed that human IFNs induced protein kinase C (PKC) in IFN-treated human neuroblastoma cells (Chatterjee et al., 1995). It will be of interest to determine in the future whether murine and Hu-α B/D IFNs activate any PKC in BALB/3T3 cells. Future experiments aimed at addressing this possibility and the possibility of involvement of other enzyme systems should facilitate in understanding a detailed mode of action of these IFNs against HSV replication in mouse 3T3 cells.

Acknowledgements

We thank Pamela Burns and Eugene Arms for excellent technical assistance and Solomon Yilma for some initial observations. We also thank Teresa Shelton for her help in preparation of this manuscript. This work was partly supported by a grant from the 'Walter-Marget-Stiftung für Infektionskrankheiten e.V.' and in part by a grant from the JCR Pharmaceuticals Company, Kobe.

References

Chatterjee, S. and Burns, P. (1990) Expression of herpes simplex virus type 1 glycoproteins in interferon-treated human neuroblastoma cells. J. Virol. 64, 5209–5213.

Chatterjee, S. and Sarkar, S. (1992) Studies on endoplasmic reticulum-Golgi complex cycling pathway in herpes simplex virus-infected and brefeldin A-treated human fibroblast cells. Virology 191, 327–337.

Chatterjee, S. and Whitley, R.J. (1989) Effect of recombinant hybrid human interferon on replication and morphogenesis of HSV-1 in monkey cells. Virus Res. 12, 33–42.

Chatterjee, S., Bradac, J. and Hunter, E. (1982) Effect of Monensin on Mason-Pfizer monkey virus glycoprotein synthesis. J. Virol. 44, 1003–1012.

Chatterjee, S., Hunter, E. and Whitley, R. (1985) Effect of cloned human interferons on protein synthesis and morphogenesis of herpes simplex virus. J. Virol. 56, 419–425.

Chatterjee, S., Nishimuro, S. and Whitley, R.J. (1990) Expression of HSV-1 glycoproteins in tunicamycin-treated monkey kidney cells. Biochem. Biophys. Res. Commun. 167, 1139–1145.

Chatterjee, S., Burns, P. and Koga, J. (1995) Effect of protein kinase C inhibitors on the antiviral activity of human alpha interferon in herpes simplex virus-infected human neuroblastoma cells. J. Virol. 69, 1315–1318.

DeStasio, P.R. and Taylor, M.W. (1990) Specific effect of interferon on the herpes simplex virus type 1 transactivation event. J. Virol. 64, 2588–2593.

Gloger, I. and Panet, A. (1984) Synthesis of herpes simplex virus proteins in interferon-treated human cells. J. Gen. Virol. 65, 1107–1111.

Greenwood, F.C., Hunter, W.M. and Glover, J.S. (1963) The preparation of I-131-labelled human growth hormone of high specific radioactivity. J. Biochem. 89, 114–123.

Horisberger, M.A. and De Staritzky, K. (1987) A recombinant human interferon-alpha B/D hybrid with a broad host-range. J. Gen. Virol. 68, 945–948.

Johnson, D.A., Gautsch, J.W., Sportsman, J.R. and Elder, J.H. (1984) Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Technol. 1, 3–8.

Koga, J., Chatterjee, S. and Whitley, R.J. (1986) Studies on herpes simplex virus type 1 glycoproteins using monoclonal antibodies. Virology 151, 385–389.

Maheshwari, R.K., Sidhu, G.S., Singh, A.K., Sivaram, S.S., Kinchington, P.R., Hay, J. and Friedman, R.M. (1994) Defective transport of herpes simplex virus glycoprotein in interferon-treated cells: role of intracellular pH. J. Interferon Res. 14, 319–324.

Meister, A., Uze, G., Mogensen, K.E., Gresser, I., Tovey, M.G., Grutter, M. and Meyer, F. (1986) Biological activities and receptor binding of two human recombinant interferons and their hybrids. J. Gen. Virol. 67, 1633–1643.

Mittnacht, S., Straub, P., Kirchner, H. and Jacobsen, H. (1988) Interferon treatment inhibits onset of herpes simplex virus immediate-early transcription. Virology 164, 201–210.

Munoz, A. and Carrasco, L. (1984) Formation of noninfective herpesvirus particles in cultured cells treated with human interferon. J. Gen. Virol. 65, 1069–1078.

Oberman, F. and Panet, A. (1988) Inhibition of transcription of herpes simplex virus immediate early genes in interferon-treated human cells. J. Gen. Virol. 69, 1167–1177.

Popik, W. and Pitha, P.M. (1991) Inhibition by interferon of herpes simplex virus type 1-activated transcription of tat-

defective provirus. Proc. Natl. Acad. Sci. USA 88, 9573–9577.

Sahni, G. and Samuel, C.E. (1986) Mechanism of interferon action. Expression of vesicular stomatitis virus G gene in transfected COS cells is inhibited by interferon at the level of protein synthesis. J. Biol. Chem. 261, 16764–16768.

Torigoe, S., Campbell, D.E., Torigoe, F., Michelson, S. and Starr, S.E. (1993) Cytofluorographic analysis of effects of interferons on expression of human cytomegalovirus proteins. J. Virol. Methods 45, 219–228.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350–4354.