Expression of Herpes Simplex Virus Type 1 Glycoproteins in Interferon-Treated Human Neuroblastoma Cells

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Human alpha interferon (IFN) significantly inhibits the replication of herpes simplex virus type 1 in human neuroblastoma cells. This inhibitory effect can be blocked by pretreatment with antiserum to IFN. We observed no significant differences in the expression of major nucleocapsid proteins, including VP5, between IFN-treated and untreated neuroblastoma cells. Electron micrographs demonstrated that there were distinct viral nucleocapsids within IFN-treated neuroblastoma cells. The expression of glycoproteins B and E was significantly reduced in these IFN-treated cells. On the other hand, glycoprotein D, although reduced in quantity, was expressed after IFN treatment. An immunofluorescence assay of the IFN-treated and virus-infected cells detected glycoprotein D in the Golgi complexes and in the nuclear membranes. Our results indicate that human alpha IFN may be useful in the study of gene expression in IFN-treated cells of neuronal origin.

Herpes simplex viruses (HSV) are among the most extensively studied animal viruses which cause asymptomatic to life-threatening diseases. Productive infections by HSV result in virus multiplication and cell death. In addition to this type of acute infection, HSV can become latent in the body of a host and often reactivates, causing serious problems. Latency is established at the level of dorsal root ganglia (1, 2). Recently, significant efforts have been made to prevent reactivation of HSV infections by treatment with antiviral drugs, although only one has had clinical success (12, 25).

Previously, we have shown that human interferons (HuIFNs) can block HSV replication in human fibroblast and monkey kidney cells (8, 11). The molecular mechanism of interferon (IFN) action on the DNA viruses, specifically on HSV, is not well understood. Reports from other laboratories have shown that HuIFNs prevent the replication of HSV (15, 20, 21), although the modes of action in various cell lines and with different types and preparations of IFNs were not identical. Furthermore, it is not known whether IFNs act in a similar fashion against HSV in cells of neuronal origin, a site for establishment of latency.

In this report, we show that human alpha interferon (HuIFN- α) inhibits the replication of HSV type 1 (HSV-1) in human neuroblastoma cells as effectively as in human foreskin fibroblast cells (8, 10).

The expression of glycoprotein B (gB) and gE was greatly reduced in IFN-treated neuroblastoma cells, as determined by immunoblot assays. On the other hand, gD, although reduced in quantity, was expressed after IFN treatment and could be detected in the Golgi complexes and nuclear membranes, indicating that intracellular transport of this glycoprotein to the site of budding occurred normally. These results suggest that IFNs might be useful in the study of gene expression in IFN-treated human cells, including cells of neuronal origin, a target for establishment of latency.

In order to determine the effect of HuIFN on the release of infectious HSV particles, neuroblastoma cells were pretreated with 0, 200, and 500 U of HuIFN- α per ml for 18 h. Human neuroblastoma cell lines were provided by T. Howard, University of Alabama at Birmingham, Birmingham.

These cells exhibited rosette formation, had microtubule arrays (as determined by morphology) and neurofilaments, and exhibited neuron-specific enolase activity (as determined by immunocytochemistry) (G. Driskill, T. Howard, D. Kelly, E. Wilson, R. Castleberry, and L. Binder, Clin. Res. 37:63A, 1989). Cells were infected with strain MP of HSV-1 (approximate multiplicity of infection, 5). The cells were then incubated at 37°C for 48 to 72 h, after which supernatants were collected and tested for their ability to form plaques in monkey kidney cells. Both concentrations of HuIFN- α blocked the replication of HSV-1 in neuroblastoma cells by more than 98% (Table 1). The block in replication was not due to toxic effects of HuIFN- α . We observed no significant changes after IFN treatment in either cell morphology or cell number. The specificity of IFN inhibition was confirmed by preincubating the IFN with antiserum to HuIFN- α and then testing its ability to block HSV-1 replication. The results demonstrated that antisera to HuIFN- α can abrogate the inhibitory effect of IFN (data not shown).

We next analyzed the status of viral proteins in IFN-treated cells to correlate the inhibition of replication with expression of virus-specific proteins, if any. Human neuroblastoma cells were pretreated with HuIFN- α and then infected with strain MP as described above. Cell lysates were collected 48 h postinfection and processed for polyacrylamide gel electrophoresis and immunoblotting. The nitrocellulose blot was reacted with a rabbit anti-HSV-1 antiserum. A separate but identical blot was incubated with rabbit antiserum to VP5. Finally, the blots were incubated with 125 I-labeled protein A after several washes. Pretreatment with IFN had no significant effect on the expression of major HSV-1 proteins, including VP5, the major capsid protein (Fig. 1B). Consistent with this observation, electron microscopy showed complete nucleocapsids within the nuclei of IFN-treated neuroblastoma cells (Fig. 2), suggesting that viral capsid proteins were synthesized in the presence of HuIFN.

Since glycoproteins are functionally needed for envelopment of nucleocapsids and budding from nuclear membranes, we examined whether the block in production of infectious virus particles was due to a defect in the expression of glycoproteins. The same cell lysates that were used in

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TABLE 1. Effect of HuIFN- α on the replication of HSV-1 in human neuroblastoma cells^a

HuIFN- α concn (U/ml)	No. of PFU/ml	% Inhibition (fold reduction)
0	8.0×10^6	0
200	1.4×10^5	98.25 (57)
500	2.2×10^4	99.70 (364)

^a Neuroblastoma cells were pretreated with HuIFN- α for 18 h and then infected with strain MP of HSV-1. HuIFN- α (approximate specific activity, 1×10^8 U/mg of protein) was provided by J. Koga, Japan Chemical Research Pharmaceuticals Co., Kobe, Japan. Strain MP of HSV-1 was provided by B. Roizman, University of Chicago, Chicago, Ill. Supernatants from treated and untreated cells were tested for their ability to form plaques in BS-C-1 cells 48 to 72 h postinfection.

the experiment described above were analyzed by immunoblotting to assess the expression of gB, gD, and gE, using monoclonal antibodies. Figure 3A shows that, unlike the levels of nucleocapsid proteins, the levels of gB and gE were greatly reduced in IFN-treated neuroblastoma cells. The inhibition of gE expression was almost complete in IFN-treated cells. On the other hand, although gD expression was reduced, this glycoprotein was expressed after IFN treatment.

Since gD was expressed in IFN-treated cells, we evaluated incorporation of glycoproteins into the extracellular viral particles (although they were very reduced in number) released from IFN-treated neuroblastoma cells. Thus, the experiment described below was performed to determine the quantities of glycoproteins in the total extracellular HSV-1 preparations from both treated and untreated cells. Cells were pretreated with IFN and then infected with strain MP as described above. Culture supernatants were collected from IFN-treated and untreated cells 48 h postinfection and clarified, and the virus was pelleted by centrifugation at

40,000 rpm for 1 h. The pellets were lysed and subjected to polyacrylamide gel electrophoresis and immunoblotting. The nitrocellulose blot was reacted with monoclonal antibodies to gB, gD, and gE. The results of this experiment showed that very little gB was expressed on the extracellular virus particles released from IFN-treated neuroblastoma cells (Fig. 3B). Furthermore, the viral particles released from treated cells almost totally lacked gD and gE. In parallel, one set of pellets was processed for a dot blot assay in order to determine the total levels of extracellular viral particles released. The nitrocellulose blot was reacted with rabbit antiserum to HSV-1. It is clear that detectable quantities of particles (14 and 18% as determined by gamma counting) were released from cells treated with IFN (200 and 500 U/ml, respectively) (Fig. 4). In addition, electron microscopic observations demonstrated the release of extracellular HSV-1 particles from IFN-treated cells (data not shown).

Although extracellular viral particles lacked gD, we showed that IFN-treated neuroblastoma cells expressed appreciable amounts of gD (Fig. 3A). However, it was not possible to determine the location of this glycoprotein inside the IFN-treated cells or the reason for the possible block in incorporation of gD into viral particles by immunoblotting. An indirect immunofluorescence experiment was performed to detect the location of gD in cells treated with IFN (200 and 500 U/ml). The immunofluorescence assay confirmed the presence of gD within the IFN-treated cells (Fig. 5). The location of gD inside the treated cells was also determined by rhodamine labeling, which indicated that there was precise expression of gD in Golgi complexes, as we found in untreated cells (Fig. 5). In addition, perinuclear staining of gD in IFN-treated cells was also prominent (Fig. 5), suggesting that gD is present on nuclear membranes.

HSV establish latency at the level of dorsal root ganglia and often reactivate, causing both asymptomatic and symptomatic infections. Previously there were no reports concerning the activity of IFNs on replication of HSV in human neuronal cells. In this study, we demonstrated that HuIFN- α blocks the replication of HSV-1 in human neuroblastoma cells. The presence of HSV nucleocapsids in IFN-treated neuroblastoma cells and the expression of nucleocapsid proteins, including VP5, indicate that in this system, IFN blocks virus replication at a late stage in morphogenesis and supports our previous findings determined with human fibroblast cells. However, the expression of HSV glycoproteins in IFN-treated neuroblastoma cells was different than the expression observed in human fibroblast cells. We were interested in the expression of glycoproteins in IFN-treated cells as these molecules are important for various biological functions. Previously, Sahni and Samuel (24) showed that the synthesis of vesicular stomatitis virus protein G is significantly inhibited in transfected COS cells treated with IFN. Several species of virus-specific glycoproteins (gB, gC, gD, gE, gG, gH, and gI) have been detected in HSV-infected cells (3, 13, 16, 18, 19, 23), although only gB, gD, and gH are essential for virus infectivity (14). Previously, we found that the expression of gB and gD is greatly reduced in IFN-treated human fibroblast and monkey kidney cells (8, 11). However, the expression of these glycoproteins is different in neuroblastoma cells than it is in human fibroblast cells. In neuroblastoma cells, although the expression of gD was somewhat reduced, gD was expressed after IFN treatment. An immunofluorescence assay of the IFN-treated and virus-infected cells clearly detected the presence of this glycoprotein in the Golgi complexes. Furthermore, the immunofluorescence assay also demonstrated that there was perinuclear

FIG. 1. Effect of HuIFN- α on the expression of HSV-1 proteins in human neuroblastoma cells. HuIFN- α -treated and untreated neuroblastoma cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then processed for immunoblotting as previously described (8). (A) Nitrocellulose blot reacted with rabbit anti-HSV-1 antiserum (Lee Biomolecular Research Laboratories, Inc., San Diego, Calif.). Lane 1, No IFN; lane 2, 200 U of IFN per ml; lane 3, 500 U of IFN per ml. (B) Blot reacted with rabbit antiserum to VP5 provided by G. Cohen, University of Pennsylvania, Philadelphia. Lane 1, No IFN; lane 2, 200 U of IFN per ml.

FIG. 2. Electron micrographs of HSV-1 nucleocapsids in thin sections of IFN-treated and untreated human neuroblastoma cells. The procedure used for electron microscopy has been described previously (4). (A) Untreated cell. Magnification, $\times 21,750$. (B) Cell treated with HuIFN- α (500 U/ml). Magnification, $\times 33,060$. Note the distinct nucleocapsids inside the nuclei of both treated and untreated cells (arrows).

FIG. 3. Effect of HuIFN- α on the expression of HSV-1 gB, gD, and gE in human neuroblastoma cells as determined by immunoblotting. Monoclonal antibodies against gB, gD, and gE were developed in our laboratory (9, 17). (A) Expression of intracellular viral glycoproteins after IFN treatment. Lane 1, No IFN; lane 2, 200 U of IFN per ml; lane 3, 500 U of IFN per ml. (B) Expression of extracellular viral glycoproteins after IFN treatment. Lane 1, No IFN; lane 2, 200 U of IFN per ml; lane 3, 500 U of IFN per ml.

FIG. 4. Release of total extracellular virus particles from IFN-treated and untreated neuroblastoma cells. The procedure which we used is described in the text. C, Uninfected control. After autoradiography, the blot was counted with a gamma counter for quantification.

staining with antibodies to gD, which suggested that this glycoprotein was transported to the nuclear membrane of the IFN-treated cells, the budding site of the nucleocapsids. However, extracellular viral particles almost totally lack gD. There are two possible explanations for the absence of gD on

FIG. 5. Indirect immunofluorescence assay of HSV-1-infected neuroblastoma cells pretreated and untreated with HuIFN- α . Interferon-treated and untreated cells were processed for the immunofluorescence assay as described previously (6), with some modifications. All samples were reacted with monoclonal antibody to gD. (A) Untreated cells, fluorescein labeled. (B) Untreated fluorescein-labeled cells reacted with rhodamine-conjugated wheat germ agglutinin. (C) Cells treated with IFN (500 U/ml), fluorescein labeled. (D) Cells treated with IFN (500 U/ml), fluorescein labeled, and reacted with rhodamine-conjugated wheat germ agglutinin. The arrows indicate the positions of Golgi complexes.

the few extracellular particles formed. First, changes in membrane fluidity which occur in IFN-treated cells (5, 22) could be responsible since any change in the nuclear membrane may interfere with nucleocapsid budding. Second, a structural modification of gD in IFN-treated cells might have occurred such that incorporation into the virions was not possible. However, the first possibility was ruled out because although gB is expressed very little inside IFN-treated cells, this glycoprotein is incorporated into particles, indicating that there is a functionally normal nuclear membrane. Thus, a structural change in the gD molecule in IFN-treated cells might be the reason that gD cannot be incorporated into virus particles.

We also studied the expression of gE in IFN-treated neuroblastoma cells. This glycoprotein is known to be responsible for the induction of Fc-binding activity on virus-infected cells (3) and has been reported to be involved in HSV-1-induced cell fusion (9). As Fig. 3A shows, the expression of gE was greatly reduced in IFN-treated human neuroblastoma cells. Extracellular viral particles released from IFN-treated cells totally lacked this glycoprotein, as expected. It is important to note that, even in control cells, the amount of gE expressed on the virus particles was very small. It is not known whether the HSV-1 particles produced from human neuroblastoma cells expressed little gE or this was due to some other reason.

In this study we found that HuIFN- α significantly blocks HSV-1 replication in human neuroblastoma cells, which means that IFN could be useful in preventing reactivation of HSV-1 infections in human cells of neuronal origin. Our results suggest that HuIFN- α may be useful for studying HSV glycoprotein expression and transport in human neuronal and other cells. Thus, future studies with IFN should contribute to a detailed understanding of HSV morphogenesis in human cells.

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