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LOW INFECTIVITY OF VESICULAR STOMATITIS VIRUS (VSV) PARTICLES RELEASED FROM INTERFERON-TREATED CELLS IS RELATED TO GLYCOPROTEIN DEFICIENCY

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We have investigated the mechanism for the low infectivity of vesicular stomatitis virus (VSV) released from interferon (IFN) -treated cells. With 10-30 units/ml of IFN there was an approximately 5-30 fold reduction in the production of virus particles, as measured by VSV proteins; however, the infectivity of the VSV released from IFN-treated mouse L_B, JLS-V9R, or human GM2504 was drastically reduced (2 to 4 logs). The low infectivity of VSV was directly related to a deficiency in virion glycoprotein (G). IFN treatment did not change the specific infectivity of the VSV particles released by HeLa cells; their G protein was also not reduced. A further effect of IFN to reduce the amount of virion M protein appeared to be secondary and was probably not related to the reduced infectivity of VSV.

In recent years it has become increasingly evident that the antiviral activity of interferons (IFNs) is not unitary (1,2). While the discovery of the IFN-associated 2'5' oligoadenylate synthetase, endoribonuclease, and kinase provide a biochemical basis for the IFN-induced inhibition of virus directed translation (3,4) , there are reported instances of virus growth inhibition by IFN that are clearly not associated with inhibition of viral protein synthesis. The inhibition of the replication of RNA tumor viruses by IFNs was the first reported system (5,6) in which virus protein synthesis was not clearly the target of IFN action. Instead, IFN treatment seemed to inhibit the various steps in the final assembly of infectious RNA tumor viruses, including virus budding and incorporation of glycoprotein into virions (7-13). We have earlier shown (14-15) that IFN-treated L_B cells released VSV particles with low infectivity that are also deficient in VSV G

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and M protein (16). These results suggested that IFNs inhibit the replication of membrane-associated RNA viruses other than RNA tumor virus at a post-translational level. This inhibitory effect can be expressed in several ways, of which two appear to be the most important: (i) a marked reduction in the release of virus particles, as in the case of most murine leukemia viruses (MLVs) and mouse mammary tumor viruses (MMTVs) (6,7,11,13); and, (ii) the combination of a small reduction in the production, and a large reduction in the infectivity of progeny virions of MLV (12-13) or VSV (14-16).

We have studied the effect of mouse and human IFN on VSV in mouse L_B and JLS-V9R and in human GM2504 and HeLa cells. With the exception of the HeLa cell system IFN treatment induced the production of VSV particles drastically reduced in infectivity. We have further shown that the low infectivity of these VSV virions was directly related to a deficiency of VSV glycoprotein G; however, IFN treatment did not reduce the infectivity of VSV particles released from HeLa cells. Likewise viral G protein was also not reduced. The effect of IFN on inhibition of M protein appeared to be secondary, and was probably not related to the reduced infectivity of VSV particles released by IFN-treated cells.

MATERIALS AND METHODS: Cells and Their Maintenance: Mouse L_B cells were originally obtained from Prof. D. Burke, University of Warwick, England and were maintained in Eagle's MEM with 10% fetal calf serum (Gibco Laboratories); mouse JLS-V9R (clone 4) was derived by chronically infecting an established mouse bone marrow cell line (JLS-V9R) with Rauscher-leukemia virus; this cell line was maintained on McCoy's 5a medium with 10% fetal calf serum. HeLa cells were obtained from Dr. John McGowan, Department of Microbiology, USUHS, Bethesda, Maryland. GM2504, a human trisomic fibroblast cell line (Down's syndrome phenotype), was obtained from the Institute for Medical Research, Camden, New Jersey. Human GM2504 and HeLa cells were grown in Eagle's MEM with 10% fetal calf serum.

Virus: VSV, Indiana strain (originally obtained from Dr. C. Buckler, NIH), was plaque purified and passaged at low multiplicities. Virus titers were expressed in tissue culture infectious doses by monitoring cytopathic effect in microtiter plates. VSV was titered in VERO cells.

Interferon and Interferon Assays: Mouse IFN was prepared and partially purified on an antibody affinity column (17). The specific activity of this preparation was greater than 4×10^7 mouse reference units per milligram of protein. Pure human α recombinant IFN was obtained from Hoffman La Roche, Inc.

Assay for Antiviral Activity: Cells were set up in 96-wells microtiter plates and treated overnight with different IFN concentrations depending on the cell

type. IFN was then removed, the cells were washed two times, and then infected with VSV at a $TCID_{50}$ of five particles for one hour. Unadsorbed virus was removed and cells were washed and refed with fresh MEM; they were further incubated for another 20-24 hours. Supernatant fluids from replicates of wells were pooled and assayed for virus titer; inhibition of viral infectivity by IFN was calculated by subtracting the log of the virus titer in IFN-treated cells from that in untreated controls.

Analysis of VSV Proteins: Unless otherwise stated, all cells were set up in 6-wells dishes and treated with IFN (30 units/ml) overnight. Cells were then infected with VSV at a $TCID_{50}$ of five particles per cell. After one hour of adsorption, unadsorbed VSV was removed and cells were washed. Cells were labelled five hours after infection with [^{35}S] methionine (5 μCi/ml, specific activity 970.2 Ci/mmol, NEN) in low methionine MEM supplemented with 2% dialyzed calf serum, and then were incubated for 18 additional hours. Supernatants were collected and an aliquot was saved for measurement of infectivity. Virus from the supernatants was pelleted at 40,000 xg for 90 minutes and pellets were dissolved in 100 μl of 2% SDS-2% mercaptoethanol. Viral proteins were analyzed on SDS/polyacrylamide slab gels (18). The incorporation of radioactive precursor was quantified by fluorography. The gels were dried and exposed on Kodak SB5-XO-Mat film.

RESULTS AND DISCUSSION: IFN (30 units/ml) treatment inhibited the virus infectivity 100-300 fold in mouse L_B and JLS-V9R cells; however, virus particle production as measured by viral proteins was inhibited only five to ten-fold (Fig. 1). Analysis of virion proteins showed that particles released from IFN-treated cells were selectively deficient in G and M proteins. Treatments of GM2504 with human IFN (10 units/ml) reduced the virus particle yield 20-30 fold; however, the infectivity of VSV was greatly reduced (more than 1000-fold). There was, in addition a selective inhibition of G and M proteins in VSV released from IFN-treated GM2504 cells.

We also studied the effects of human IFN in HeLa cells; here, however, IFN treatment (30 units/ml) did not change the specific infectivity of VSV released from treated cells (Fig. 1). A 10-fold decrease in VSV infectivity was proportional to a 7-fold inhibition of virion production by IFN-treated cells. Furthermore, VSV G protein was also not decreased by IFN treatment, even though M protein was significantly inhibited.

Figure 2 shows the scanning profile of G, N, and M proteins in VSV released from control and IFN treated L_B, JLS-V9R, GM2504, and HeLa cells, a determination carried out in a Schimudizu TLC gel scanner. The concentrations of G and M protein were calculated on the basis of the ratio of G or M protein to N-protein concentration and were compared with the ratio of G or M to N in

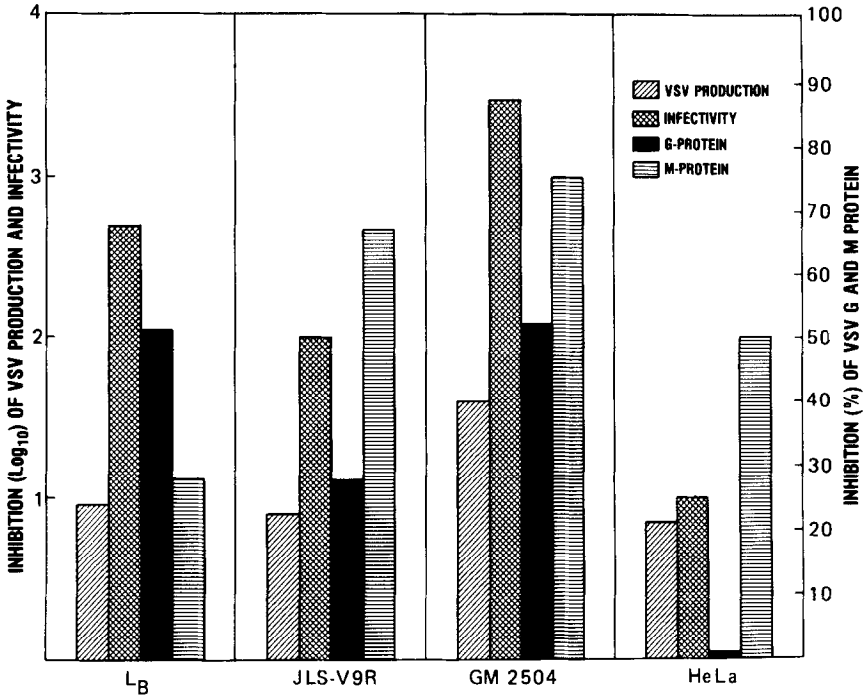

Figure 1. Comparison Between VSV Particle Production, Infectivity, and G and M Protein of VSV Released from Various Cells Treated with IFN: Cells were set up in 6-wells petri dishes and were treated with IFN overnight. All cells were then washed and infected with VSV at a $TCID_{50}$ of five particles per cell for one hour. Unadsorbed virus was removed and cells were replenished with fresh MEM and incubated for 24 hours. Supernatant fluids were assayed for virus infectivity in same cells in which the antiviral activity test was performed. In a parallel experiments VSV proteins were labelled for 18 hours, five hours post infection with [^{35}S] methionine (5 μCi/ml; specific activity 970.2 Ci/mmol, NEN, Boston) in MEM with low concentrations of cold methionine (GIBCO). Supernatants were pelleted at 40,000 xg for 90 minutes and divided into two parts. Pellets were suspended in 100 μl of 1% Triton X-100 (v/v) and an aliquot was immunoprecipitated to measure the VSV particle production by virus-associated protein. Another part (50 μl) was applied to gels for electrophoresis and fluorography. The fluorograms were scanned to quantitate the ratio of G and M protein to N protein.

controls. IFN treatment caused a 50, 27, and 53% reduction in G protein in VSV released from IFN treated L_B, JLS-V9R and GM2504 cells respectively. A similar pattern of reduction in M protein was seen where IFN caused 30, 65, and 75% inhibition in M protein of VSV released from L_B, JLS-V9R and GM2504 cells respectively. In HeLa cells, however, IFN did not cause a significant reduction in the G-protein, even though a significant reduction (50%) in M protein was seen.

These results indicated that IFN treatment of mouse L_B, JLS-V9R, or human GM2504 reduced VSV particle production 5 to 30-fold; however, the infectivity of these particles was drastically inhibited (100-3000 fold). These results

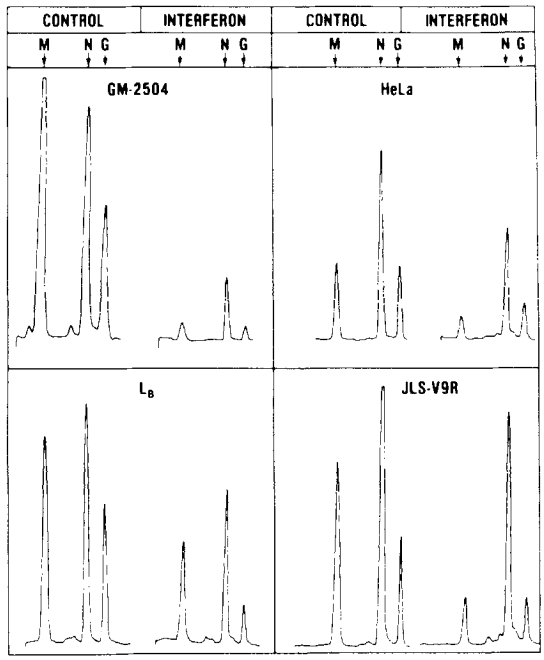

Figure 2. Scanning profiles of VSV G, N and M proteins in virus released from control (untreated) and IFN treated L_B, JLS-V9R, GM2504 and HeLa cells.

suggested that in the presence of IFN not only was the production of infectious VSV particles inhibited, but also the production of non-infectious particles was disproportionately enhanced. VSV particles released from these cells were selectively deficient in G and M protein; however, treatment of HeLa cells with human IFN did not change the specific infectivity of VSV particles produced. IFN caused an inhibition in virion production by 7 to 8-fold; the inhibition in virus infectivity (10-fold) closely paralleled the inhibition of virus yield. Analysis of proteins on gels showed that G protein production was not inhibited in VSV released from HeLa cells as compared to VSV released by L_B, JLS-V9R or GM2504 cells, where IFN selectively inhibited the G protein. Probably as a consequence of these VSV particles with drastically reduced infectivity were produced. M protein was, however, significantly inhibited in VSV released from HeLa cells suggesting that the effect of IFN on M protein was not related to a decrease in particle infectivity; therefore, the drastic reduction in infectivity of VSV released from L_B, JLS-V9R or GM2504 can probably be directly attributed to the deficiency in G protein.

These results resemble those reported in TB cells infected both de novo and chronically with MLV. Our current findings suggest that this mechanism of action of IFN may not be restricted to RNA tumor viruses. Although the precise mechanism for the inhibitory effect of IFN on RNA tumor virus and VSV is not understood, it may well be related to the reduced amount of glycoproteins in the virions produced. Since previous studies clearly show that VSV particles with a reduced amount of G protein are low in infectivity (20,21), it is very likely that at least some of the reduced infectivity of VSV and MLV particles is due to the presence of reduced amount of glycoprotein. It is possible that IFN-induced changes in the cell plasma membrane may be the basis for the alterations in infectivity of both VSV and MLV produced after IFN treatment, since these viruses bud from the cell surface (1).

Recently, Olden et al. (22) reported a 10-20-fold inhibition of VSV production with unchanged specific infectivity in L-cells treated with 30 units of IFN. The particles released from IFN-treated cells were not deficient in G or M proteins. The apparent discrepancy between Olden's et al. (22) and our present results can be directly related to the relative IFN insensitivity of their cells. With 30 units/ml of IFN they reported a 10-20 fold inhibition of the production of infectious VSV which corresponded to a 10-20 fold reduction in virus particle production. With 30 units/ml of IFN we regularly see a 200-600 fold inhibition of infectious VSV and only a 10-fold reduction in virion production as measured by VSV associated RNA, nucleocapsid protein, or transcriptase.

Faltynek and Baglioni (23) reported that treatment of HeLa cells had no effect on the glycosylation of viral and cellular proteins. VSV released from control and IFN-treated HeLa cells was found to be equally glycosylated. Treatment of HeLa cells with IFN showed an inhibition of incorporation of N-acetylglucosamine into dolichol derivatives in a cell-free assay. These authors attributed this effect of IFN in part to a high nucleotide pyrophosphatase activity, that degraded the sugar nucleotide substrate rather than to an effect on glycosylation in HeLa cells; however, they did not provide data

on virus infectivity or on the G and M protein content of the VSV produced by HeLa cells. In this paper we have studied the effect of human α recombinant IFN on VSV replication in HeLa cells; the results showed that IFN did not change the specific infectivity of VSV released from the IFN-treated cells. The reduction in infectivity closely followed the reduction in virion production in HeLa cells. Since no selective reduction in VSV G protein was seen, one would not expect glycosylation to be significantly affected.

We emphasize that it is meaningless to study the effect of IFN on VSV G-protein glycosylation if IFN treatment does not induce a deficiency in virion G protein and hence a decrease in the specific infectivity of the VSV produced. Therefore, it is most unlikely that Olden et al. (22) and Faltynek and Baglioni (23) could have seen differences in the glycosylation of VSV G protein after IFN treatment, because neither a decrease in specific infectivity, nor a deficiency in G protein was demonstrated in the systems used by them. Our unequivocal findings of a reduction in G and M protein in VSV produced by IFN treated mouse L_B cells has been confirmed by Jay et al. (24) and also by several groups in infection with murine leukemia (MLV), another membrane associated virus (25-26). These studies report that MLV deficient in glycoprotein gp 69/71 was produced by IFN-treated cells. Another study (27) reported inhibition of the rate of virus protein glycosylation in IFN treated cells infected with MLV.

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