volves an initial hydroxylation of tyrosine to dopa within synaptosomes. Subsequent decarboxylation of the dopa is catalyzed by endogenous decarboxylase and requires the presence of synaptosomes. The concentration of tyrosine used is saturating for catecholamine synthesis (K_m, 1 μM) but not for tyrosine uptake (K_t, 15 μM) under these conditions.
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Selective Inhibition of Glycoprotein and Membrane Protein of Vesicular Stomatitis Virus from Interferon-Treated Cells

Abstract. *A 200-fold inhibition in the titer of infectious vesicular stomatitis virus (VSV) was produced in cultures of Ly cells treated with 30 reference units of interferon per milliliter. Virus particle production, as measured by VSV particle-associated transcriptase, or nucleocapsid protein was inhibited by a maximum of tenfold. The glycoprotein and membrane protein content was reduced in VSV derived from interferon-treated cells. Thus interferon-treated cells may have produced VSV particles with low infectivity, which may be related to the reduced amount of glycoprotein incorporated into such particles. These findings resemble those reported in interferon-treated cells infected with murine leukemia viruses.*

Interferon (IF) inhibits the replication of many viruses. In most systems studied, virus-directed translation or transcription was inhibited (*1*); however, an unusual inhibitory effect of IF treatment on the replication of RNA tumor viruses has been described (*2*). Inhibition of virus production did not correlate with inhibition of the accumulation of viral RNA (*3*) or of viral proteins (*4*). In IF-treated AKR cells production of both endogenous murine leukemia virus (MLV) particles and infectious MLV is decreased, although the intracellular concentration of viral p30 antigen is increased (*4*). Studies on the action of IF in Moloney murine leukemia virus (MMLV) infection of mouse bone marrow and thymus (TB) cells showed that in IF-treated cells there was a 2000-fold decrease in the production of infectious MMLV, a 10- to 20-fold decrease in virus-specific extracellular reverse transcriptase activity, and only a twofold difference in the number of budding viral particles observed on the plasma membrane as determined by scanning electron microscopy (*2*, *5*). In JLS-V5 cells treated with IF the number of cell-associated viral particles was increased, while the infectivity/particle ratio was reduced (*6*). These results suggested that in these systems the release of virus from the plasma membrane or the production of infectious virus particles was inhibited by IF treatment.

One question about this work is whether such findings are restricted to RNA tumor virus systems. We have studied the effect of low concentrations of IF (3 to 30 U/ml) on vesicular stomatitis virus (VSV) infection of Ly cells, in contrast to previous studies, where the IF concentration was higher and VSV-directed transcription or translation was inhibited (*7*). Our results indicated that in Ly cells treated with IF (30 reference units per milliliter) there was an approximately 200-fold reduction in the titer of infectious VSV particle production; however, virus particle production as measured by virion-associated transcriptase or N protein (the structural protein present in VSV in the highest concentration) was inhibited by a maximum of tenfold at this concentration of IF. However, we observed a marked inhibition in glycoprotein (G) and membrane or matrix (M) protein of VSV released from IF-treated cells.

The Indiana strain of VSV (originally obtained from C. Buckler) was plaque-purified and passaged at low multiplicities. Virus titer was assayed as 50 percent tissue culture infectious doses ($TCID_{50}$) by the cytopathic effect (CPE) in BHK or Vero cells with the use of microtiter plates; the titer was 2×10^9 $TCID_{50}$ per milliliter. The Ly mouse cell line (obtained from J. Youngner) is highly sensitive to mouse IF. Mouse IF was prepared and partially purified on an antibody affinity column (*8*). The specific activity of the preparation was greater than 4×10^7 mouse reference units per milligram of protein.

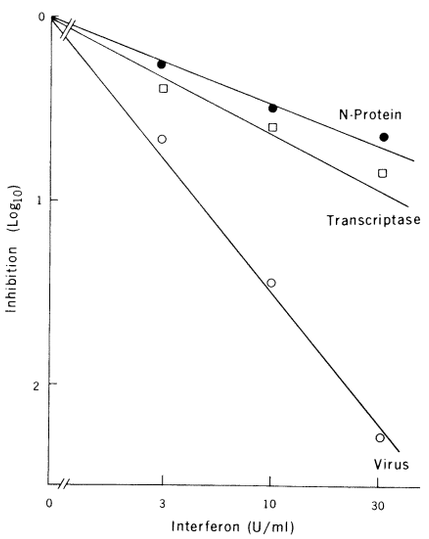

Fig. 1. Effect of IF on virus infectivity, transcriptase, and nucleocapsid (N) protein of released VSV: Ly cells were grown in 150-cm^2 flasks (2×10^7 cells) and treated with 0 to 30 units of IF for 14 hours; the cell monolayers were washed three to four times to remove the residual IF. All the monolayers were then infected with VSV at a multiplicity of five infectious particles per cell and incubated overnight. The supernatants were collected and sedimented at 10,000*g* for 30 minutes to remove cell debris. Portions were saved for infectivity, and the remainder was sedimented at 42,000*g* for 2 hours. The pellets were washed two to three times and used for transcriptase assay (*13*). To study the virion proteins, Ly cells were treated with IF as described above. After virus adsorption and incubation for 5 hours, the monolayers were washed with leucine-free medium and then [^{3}H]leucine (15 to 20 μCi/ml, specific activity 121.1 Ci/mmole) was added to monolayers in special MEM (minimum essential medium) devoid of unlabeled leucine and serum. After 3 hours of incubation, 2 ml of MEM supplemented with 2 percent fetal calf serum was added to each culture and cells were incubated overnight. The supernatants were collected, and virus was centrifuged at 42,000*g* for 2 hours. The pellet was suspended in small volumes of TNE (tris, NaCl, and EDTA) buffer (*p*H 7.2) and virus was further purified by banding on 20 to 60 percent (by weight) equilibrium sucrose gradients for 16 hours at 72,000*g*. Proteins were analyzed by polyacrylamide gel electrophoresis (*14*).

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Infectious virus titer in virus samples from cells treated with IF at 3 to 30 U/ml was reduced 50- to 250-fold (Fig. 1); virus particle production as measured by transcriptase activity or N protein was inhibited by a maximum of tenfold.

Purified virus samples with the same amount of radioactivity were applied on gels and subjected to electrophoresis. The gels were then sliced and the incorporation of radioactive precursor in each fraction was quantified. Results show a selective inhibition of G and M protein of VSV derived from IF-treated cells. Virus samples with the same amount of radioactivity were also analyzed on sodium dodecyl sulfate polyacrylamide slab gels, and the incorporation of radioactive precursors were quantified by fluorography (*9*). Four proteins (G, N, NS, and M, respectively) were present in samples of virus not treated with IF; however, in virus from IF-treated cells, there was a marked inhibition in G and M protein. The migration of protein was similar in both of the samples (Fig. 2).

These findings suggested that in the presence of IF not only is the production of infectious virus particles inhibited, but also the production of noninfectious virus particles is disproportionately enhanced. The results in many respects resemble those previously reported (*2*) in IF-treated cells infected with MLV. We concluded that such findings are not limited to murine RNA tumor virus systems.

The inhibition of membrane-associated virus yields by treatment with low concentrations of IF may be closely related to functional abnormalities in the protein incorporated into the virion or to absence of specific protein in the noninfectious virions produced (*2*). Since many studies (*10*) have demonstrated that VSV particles with reduced amount of glycoproteins are low in infectivity, it is likely that at least some of the reduced infectivity of VSV particles produced by IF-treated cells may be due to the reduced amount of this protein incorporated into such particles. It is possible that induced changes which have been reported to occur in the plasma membrane of IF-treated cells (*11*) may account for the alteration in infectivity of both VSV and murine RNA tumor virus, since these viruses bud from the cell surface as a terminal step in the replication process.

The noninfectious particles produced by IF-treated cells appear to contain only 42*S* viral RNA; they do not interfere with the growth of wild-type VSV, nor do they produce IF in culture. They are, therefore, unlikely to be defective interfering forms of VSV. Such noninfectious forms may, however, play a role in the initiation of chronic infections by VSV in IF-treated cells (*12*).

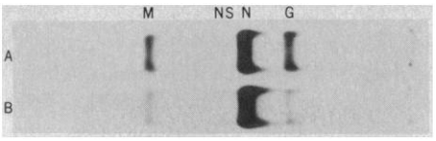

Fig. 2. Fluorograms of purified VSV proteins with equal amounts of radioactivity (15,000 count/min) applied to each gel: The gels were subjected to electrophoresis and then stained with Coomassie brilliant blue R. The gels were destained and impregnated with 2,5-diphenyloxazole (New England Nuclear), dried, and exposed to Kodak X-Omat x-ray film. Phosphorylase B (92,500 daltons), bovine serum albumin (69,000 daltons), ovalbumin (46,000 daltons), carbonic anhydrase (30,000 daltons), and cytochrome c (12,300 daltons) were used as reference protein molecular markers. (A) proteins of VSV released from cells not treated with IF; (B) proteins of VSV released from cells treated with IF (30 reference units per milliliter). *NS*, protein associated with the VSV transcriptase complex.

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Red Blood Cells: Their Dual Role in Thrombus Formation

Abstract. *Red blood cells may have a physical and chemical effect on the interaction between platelets and blood vessel surfaces. Under flow conditions in which primarily physical effects prevail, platelet adhesion increases fivefold as hematocrit values increase from 10 to 40 percent but undergoes no further increase from 40 to 70 percent, implying a saturation of the transport-enhancing capabilities of red cells. For flow conditions in which platelet-surface reactivity is more dominant, platelet adhesion and thrombus formation increase monotonically as hematocrit values increase from 10 to 70 percent. Thus red cells may have a significant influence on hemostasis and thrombosis; the nature of the effect is apparently related to the flow conditions.*

The accepted sequence of events in the formation of a platelet thrombus—platelet adhesion, release of platelet metabolites, and growth and stabilization of cellular masses consisting predominantly of platelets—generally attributes little role to the erythrocytes. However, abnormalities in red blood cell concentration in the absence of a demonstrated platelet abnormality can result in prolonged bleeding that is correctable by transfusion of red cells (*1*).

In flowing blood, overall platelet interaction with the subendothelium depends on two independent mechanisms: (i) transport of platelets from the blood to the vessel wall and (ii) reaction of the platelets with the vascular components. Platelet transport is governed by purely physical factors, namely the platelet diffusion coefficient and the blood shear rate at the vessel wall. Platelet-subendothelium reactivity is predominantly determined by chemical factors. Hellem (*2*) and Gaarder *et al.* (*3*) originally proposed that red cells play a chemical role by releasing adenosine diphosphate (ADP), a potent platelet-aggregating agent. Conversely, Turitto and Baumgartner (*4*) suggested that increased platelet adhesion and thrombus formation on subendothelium exposed to whole blood could be entirely accounted for by the increased rate of platelet transport to the surface rather than enhanced platelet-surface reactivity.

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