Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 835–839, February 1982
Cell Biology

Interferon inhibits Sendai virus-induced cell fusion: An effect on cell membrane fluidity

(paramyxovirus/syncytium formation/diphenylhexatriene polarization/cell plasma membrane stiffening)

SUBHENDRA CHATTERJEE*, HERBERT C. CHEUNG†, AND ERIC HUNTER*

*Department of Microbiology and †Department of Biomathematics, University of Alabama, University Station, Birmingham, Alabama 35294

Communicated by Peter K. Vogt, October 9, 1981

ABSTRACT **Interferon can affect several cellular functions, in addition to its antiviral activity. We report here that pretreatment of human cells with homologous interferon significantly inhibits cell fusion induced by Sendai virus and that this refractory state is accompanied by a decrease in cell plasma membrane fluidity. Multinucleate cell formation induced by β-propiolactone-inactivated Sendai virus in human fibroblast cells (a system in which fusion results from an interaction of the viral glycoprotein with the cell membrane) was inhibited by more than 90% after addition of human interferon for 18–24 hr. This inhibition could be neutralized by antiserum to interferon. Furthermore, inhibitor studies with cycloheximide and actinomycin D clearly indicated that synthesis of protein and RNA is necessary to establish the resistant state. To determine whether the inhibition of Sendai virus-induced cell fusion resulted from interferon-induced changes at the cell plasma membrane, experiments were carried out using the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene, which is capable of sensing molecular motions in the hydrocarbon core of the bilayer structure. A significant decrease in the membrane fluidity of interferon-treated cells was observed. It is likely, therefore, that the inhibitory effect on Sendai virus-induced cell fusion observed in interferon-treated cells results from an increased rigidity of the target cell membrane.**

In addition to their potent antiviral action, interferons (IFNs) have various effects on normal cellular functions. It can bind to specific receptors in the plasma membrane of normal and transformed cells and regulate both their motility (1, 2), and their rates of growth and division (3, 4). IFN treatment has also been shown to alter both plasma membrane density and the concentrations of some plasma membrane glycoproteins (5–7). Furthermore, it is known that IFN plays an important role in modulating the immune response of a host to infection (8, 9). It is clear that many of these changes induced by IFN could be mediated by alterations in membrane-associated functions of the treated cell.

We showed previously that pretreatment of cells with homologous IFN reduced the number as well as the size of multinucleate cells induced after infection with the type D retrovirus M-PMV (10). These results were consistent with our findings that cell fusion induced by M-PMV and two other type D retroviruses required penetration of the cell by the virus and translation of virus-associated RNA (11, 12). Studies from several laboratories had indicated that translation of viral mRNA could be severely inhibited in IFN-treated cells (reviewed in refs. 13 and 14). This inhibition appears to occur via two discrete pathways: the first involves phosphorylation of eukaryotic initiation factor 2 which might prevent the binding of tRNA to 40S ribosomal subunits (15), and the second is through a 2′,5′ A-dependent endonuclease which is activated in IFN-treated cells (16, 17).

Despite the fact that the inhibition of M-PMV-induced fusion might be correlated with a block in translation, it could not be excluded that the inhibition of syncytium formation resulted from IFN-induced changes at the cell surface. To differentiate between these two possibilities, experiments have been carried out with inactivated Sendai virus as a fusing agent. Cell fusion induced by this virus results from a direct interaction of viral glycoprotein with receptors in the cell plasma membrane. In this type of cell fusion, commonly referred to as "fusion from without" (18), the interaction of surface proteins of the virus with the cell membrane is sufficient to induce cell-to-cell fusion (19), and no virus-specific metabolism is necessary. The studies reported here clearly show that treatment of human cells with homologous interferon decreases their susceptibility to fusion by Sendai virus and results in a decreased fluidity of the cell plasma membrane.

MATERIALS AND METHODS

Cell Cultures and Virus. Primary human foreskin cells (HFS) and normal rhesus monkey fetal lung cells (DBS-FRHL-2) were grown as described (12). Detroit 550 cells, a human fibroblast skin cell line obtained from the American Type Culture Collection, was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal serum and 0.05% sodium bicarbonate. All the sera used were heat inactivated. Purified β-propiolactone-inactivated Sendai virus was prepared as described (11).

Chemicals and Reagents. Cycloheximide, actinomycin D, and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma. Human lymphoblastoid IFN ($>1 \times 10^6$ National Institutes of Health units/mg of protein) was kindly provided by C. B. Anfinsen. Human foreskin fibroblast IFN (1.9×10^6 National Institutes of Health units/mg of protein) was obtained from Collaborative Research (Waltham, MA). Antiserum (anti-hIFN) prepared against highly purified human fibroblast (β) IFN and which precipitates a single polypeptide in *in vitro* translation studies (20) was kindly provided by P. M. Pitha. This antiserum has a titer of 50,000 units/ml (1 unit neutralizes 1 National Institutes of Health unit of human foreskin fibroblast IFN).

Assay for Cell Fusion. In general, semiconfluent or confluent monolayers of human and rhesus monkey cells were treated with either human lymphoblastoid or human fibroblast IFN for 18–24 hr at 37°C. In some experiments, cells were treated with cycloheximide or actinomycin D in addition to interferon treatment. One set of cells was kept as an untreated control. Both treated and untreated cells were then infected

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: IFN, interferon; DPH, 1,6-diphenyl-1,3,5-hexatriene; anti-hIFN, antiserum (antibody) to human fibroblast IFN; HA, hemagglutination; P_i/NaCl, phosphate-buffered saline.

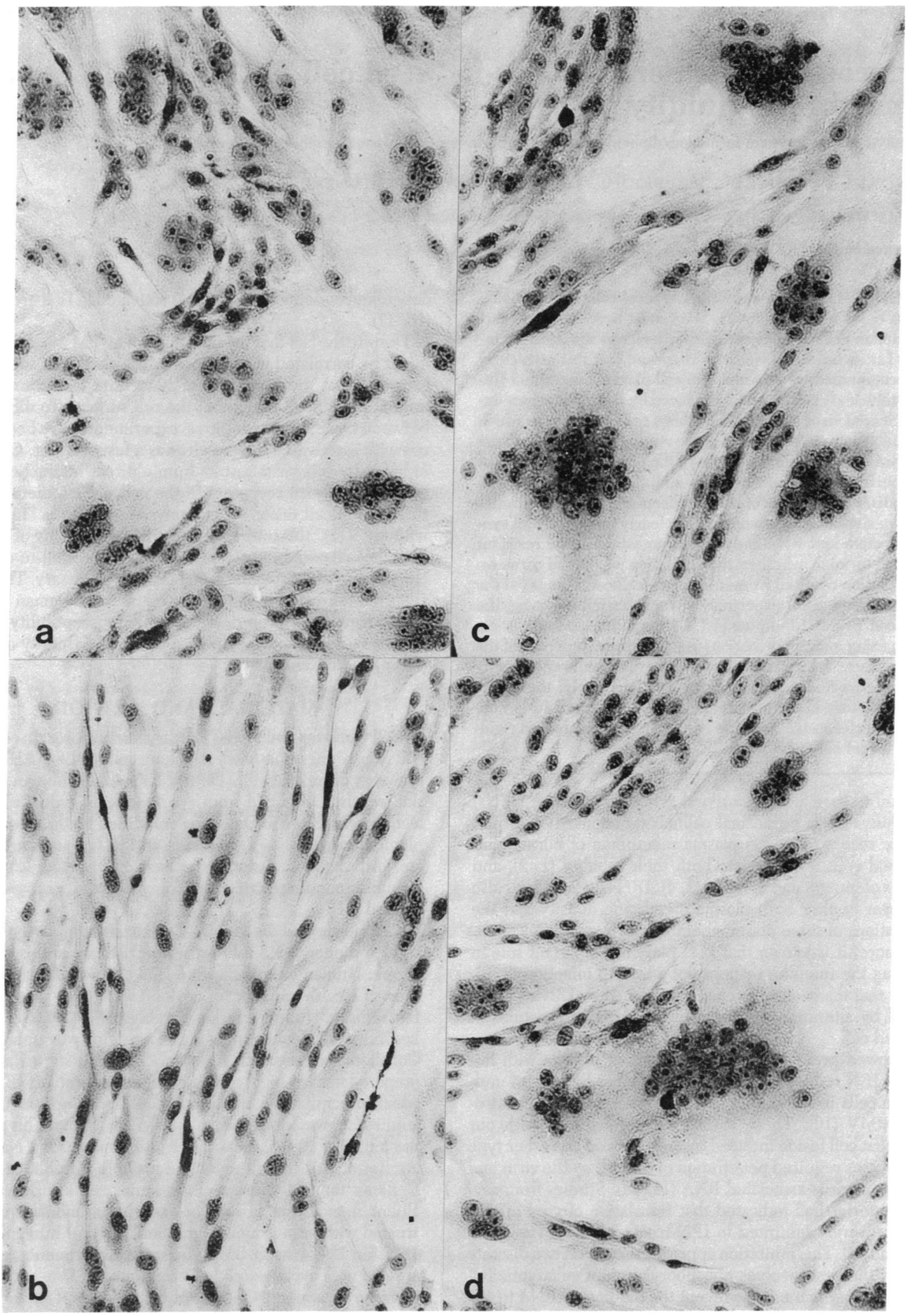

FIG. 1. Effect of human lymphoblastoid IFN on multinucleate cell formation induced by Sendai virus in human foreskin and rhesus monkey fetal lung cells. Semiconfluent monolayers of human foreskin fibroblast cells and normal rhesus monkey fetal lung cells (DBS-FRHL-2) were pretreated with human lymphoblastoid IFN (70 units/ml) for 24 hr at 37°C. One set of human cells and one set of rhesus monkey cells were kept as untreated controls. Both treated and untreated human and monkey cells were then infected with 1000 HA units of β-propiolactone-inactivated

with 1000 hemagglutination (HA) units of β-propiolactone-inactivated Sendai virus. After incubation for 2–3 hr at 37°C, the cells were stained with May–Grunwald–Giemsa stain as described (11). The number of syncytia per cm^2 in each group was determined and the percentage inhibition of syncytium formation was calculated.

Neutralization of Human IFN. Human fibroblast IFN (100 units/ml) was mixed with an appropriate volume of anti-hIFN, incubated at 37°C for 30 min, and added to the human foreskin cells. In parallel, equal volumes of human fibroblast IFN and anti-hIFN were incubated separately at 37°C for 30 min and then added to the human cells as controls. All the cells were incubated for 18–24 hr at 37°C. Cells were then infected with 1000 HA units of β-propiolactone-inactivated Sendai virus and processed as above.

Treatment of Human Cells with DPH. Human foreskin cells were pretreated with human foreskin fibroblast IFN at 10 and 100 units/ml 48 hr at 37°C. One set of human cells was kept as untreated control. Cells were then trypsinized and resuspended in phosphate-buffered saline (P_i/NaCl) at pH 7.2. Freshly prepared 6 μM DPH (prepared by injecting 2 mM DPH dissolved in tetrahydrofuran into P_i/NaCl) was mixed with an equal volume of cells suspended in P_i/NaCl. The resulting mixture was incubated at 37°C for 15–20 min and then brought to and held at 20.0 ± 0.1°C for polarization measurement in a Perkin–Elmer 650-40 ratio fluorescence spectrometer (λex = 339 nm; λem = 490 nm; band width = 20 nm). Final conditions: DPH, 3 μM; cells, 4×10^5/ml.

The degree of polarization was computed from $p = (F_\parallel - F_\perp)/(F_\parallel + F_\perp)$, in which $F_\parallel$ and $F_\perp$ are the fluorescence intensities of the vertically and horizontally polarized components, respectively, with excitation vertically polarized. The polarization value for untreated cells was 0.305 ± 0.010. This value determined from several preparations varied by no more than 5%.

RESULTS

Sendai Virus-Mediated Cell Fusion Is Inhibited by IFN. To determine the effect of human IFN on Sendai virus-induced cell fusion, human foreskin cells were pretreated with human lymphoblastoid IFN at 70 units/ml for 24 hr (IFN units are expressed as National Institutes of Health reference units). Cells were then infected with β-propiolactone-inactivated Sendai virus (1000 HA units) and stained by the May–Grunwald–Giemsa technique 2–3 hr later. Pretreatment of these human cells with homologous IFN blocked the fusing ability of Sendai virus almost completely. Few syncytia were observed in the IFN-treated cells in contrast to untreated cultures (Fig. 1 *a* and *b*). Furthermore, those that were formed were significantly smaller than those in untreated cells and rarely exceeded three or four nuclei per cell. In order to rule out the possibility that the effect of IFN was unique to the source of IFN and to the cell type used, this experiment was repeated with human fibroblast IFN and human foreskin cells and with both types of IFN and Detroit 550 cells, a human fibroblastic cell line. In every case a similar level of inhibition of Sendai virus-induced cell fusion was observed (Tables 1 and 2). Thus, this property of IFN is not restricted to a particular IFN type or target cell.

On the other hand, pretreatment of rhesus monkey fetal lung cells with the same concentration of human IFN had essentially no effect on Sendai virus-induced cell fusion (Fig. 1 *c* and *d*). This is consistent with our observation that, even at 200 units/ml, human fibroblast or lymphoblastoid IFN does not completely protect these cells from vesicular stomatitis virus infection (ref. 10; unpublished data). This result might be expected from the species specificity of IFN preparations although these particular cells may be more refractory to human IFN; more importantly, it rules out the possibility of a nonspecific inhibitory factor in the IFN preparations. These results show that the addition of human IFN alters the susceptibility of human cells to Sendai virus-induced fusion and suggest that the surface membrane of such treated cells has been altered.

Abrogation of IFN Effects by Antiserum and Metabolic Inhibitors. The above experiments strongly suggested that the observed inhibition of Sendai virus-induced fusion is a general property of both fibroblast and lymphoblastoid IFNs. Experiments to confirm the specificity of this phenomenon were carried out.

Preincubation of human fibroblast IFN with an excess of anti-β-IFN antiserum almost completely abolished the inhibitory effects observed in control plates treated with IFN that had been incubated alone (Table 1). The abrogating effect of the anti-β-IFN antiserum was not observed in control experiments in which it was mixed in an identical manner with lymphoblastoid IFN (data not shown). This is consistent with the finding that anti-hIFN shows only marginal crossreactivity (<0.01%) with human leukocyte IFN preparations (21).

To confirm that the observed inhibition of Sendai virus-induced fusion required the synthesis of effector macromolecules and possibly the establishment of an antiviral state, rather than mere binding of IFN to cells, experiments utilizing metabolic inhibitors were carried out. It is known that cycloheximide, an inhibitor of protein synthesis, and actinomycin D, which inhibits RNA synthesis, both block the establishment of an antiviral state in cells that are treated simultaneously with IFN (22, 23). In initial experiments, confluent monolayers of Detroit 550 cells were pretreated with human lymphoblastoid IFN (200 units/ml, for 18–24 hr) alone or together with cycloheximide (1 μg/ml) as shown in Table 2. Cells were then infected with β-propiolactone-inactivated Sendai virus and stained 2–3 hr afterward. In a second experiment, human foreskin fibroblast cells were pretreated with human fibroblast IFN (100 units/ml, for 18–24 hr) alone or together with cycloheximide or actinomycin D as shown in Table 2. The results of these experiments clearly demonstrate that the inhibitory effect of IFN is abrogated by both drugs, strongly indicating that the action of IFN on Sendai virus-induced cell fusion requires *de novo* synthesis of both RNA and protein and possibly the establishment of an antiviral state in these cells.

Reduction of Membrane Fluidity in IFN-Treated Cells. Because Sendai virus-induced early fusion results from an interaction between the viral glycoproteins and the plasma membrane of the target cell, it seemed most likely that the inhibition of the fusion observed in these experiments was due to a change in the nature of the plasma membrane itself. Experiments were therefore carried out to determine whether treatment of cells with IFN resulted in an altered fluidity of the plasma membrane. In these experiments, we used, as fluorescence probe, DPH which is capable of sensing molecular motions in the hydrocarbon core of the bilayer structure and thus can indicate changes in the fluidity of the plasma membrane. DPH previously has been used to investigate membrane fluidity in normal

Sendai virus. After incubation for 2–3 hr at 37°C, the cells were stained by the May–Grunwald–Giemsa method (11). (×480.) (*a*) Normal human foreskin cells infected with Sendai virus, showing numerous syncytia. (*b*) IFN-pretreated human foreskin cells, showing normal form of the fibroblast cells after Sendai virus infection. No syncytia can be observed. (*c*) Normal rhesus monkey fetal lung cells infected with Sendai virus. Several large multinucleate cells can be observed. (*d*) IFN-treated rhesus monkey fetal lung cells, also showing numerous large syncytia.

Table 1. Neutralization of the antiviral effect of human fibroblast IFN by anti-hIFN

Addition	% inhibition of syncytium formation
None	0
IFN (100 units/ml)	81.5
Anti-hIFN	0
IFN + anti-hIFN	5.7

Semiconfluent monolayers of human foreskin fibroblast cells were pretreated with human foreskin fibroblast IFN or anti-hIFN (at 100 units/ml) or a preincubated mixture of both for 18–24 hr [2 units of the unreacted IFN decreased vesicular stomatitis virus plaque formation by more than 50% in these cells (data not shown)]. Cells were then infected with β-propiolactone-inactivated Sendai virus and stained.

and transformed lymphocytes (24), in lymphocytes obtained from diabetic and control subjects (25), and in other cell types (26). In the current experiments, human foreskin fibroblast cells were pretreated with 10 and 100 units of human foreskin fibroblast IFN for 48 hr, at which time the cells were trypsinized, resuspended in P_i/NaCl, and processed to determine the polarization values.

The polarization value of the IFN-treated sample was increased significantly compared to that of an untreated control (Fig. 2). The increased polarization could be caused by either a change in the excited state lifetime of the incorporated probe or a decrease in the rotational mobility of the probe in the treated cells. The latter alteration would imply a decrease in membrane lipid fluidity. To distinguish between these two possibilities, the lifetime of the probe incorporated into both untreated and treated cells was determined in a photon-counting PRA 2000 pulse nanosecond fluorimeter using 339 nm for excitation and a Corning 3-73 cutoff filter to isolate the emission. The decay data were analyzed as described (27). Two lifetimes were detected with both samples, one in the range 3–4 nsec and the other around 9 nsec. The weighted average (± SD) was 7.9 ± 0.91 nsec for the untreated sample and 8.30 ± 0.55 nsec for

Table 2. Effect of human IFN, cycloheximide, and actinomycin D on Sendai virus-induced fusion of human cells

Addition	% inhibition of syncytium formation
Exp. 1*	
None	0.0
IFN (200 units/ml)	94.0
Cycloheximide (1 μg/ml)	4.0
IFN + cycloheximide	12.0
Exp. 2†	
None	0.0
IFN (100 units/ml)	82.0
Cycloheximide (1 μg/ml)	0.0
Actinomycin D (0.1 μg/ml)	0.0
Cycloheximide + IFN	8.0
Actinomycin D + IFN	6.0

* Confluent monolayers of Detroit 550 cells were pretreated with human lymphoblastoid IFN alone or together with cycloheximide for 18–24 hr at 37°C. One set of cells was kept as an untreated control. Both treated and untreated cells were then infected with β-propiolactone-inactivated Sendai virus and stained.

† Semiconfluent monolayers of human foreskin fibroblast cells were pretreated with human foreskin fibroblast IFN alone or together with cycloheximide or actinomycin D for 18–24 hr at 37°C. One set of cells was kept as an untreated control. Both treated and untreated cells were then processed as above.

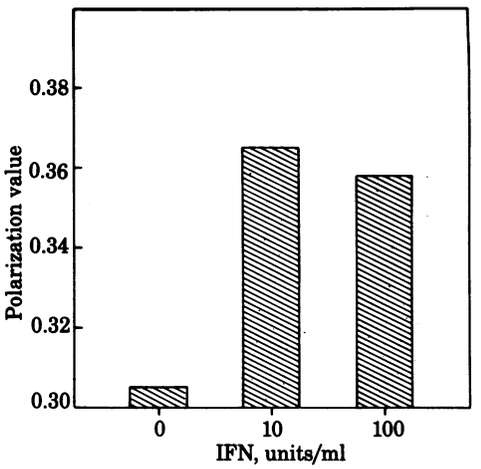

FIG. 2. Fluorescence polarization of DPH in human foreskin fibroblast cells with and without treatment with human foreskin fibroblast IFN for 48 hr at 37°C. One set of human cells were kept as an untreated control.

the treated sample. The difference cannot be considered significant. This result suggests that the increased polarization values observed after IFN treatment reflect a more rigid membrane structure. It is likely therefore that the inhibitory effect on Sendai virus-induced fusion observed in IFN-treated cells results from a decrease in the fluidity of the target cell plasma membrane.

DISCUSSION

In this report we have shown that pretreatment of human cells with homologous IFN renders them refractory to Sendai virus-induced cell fusion. Because this phenomenon was observed in two different cell types with different IFN preparations from two different sources, it is not restricted to a single type of cell or IFN. Moreover, it suggests that this refractory state is a common cellular response to both α and β types of IFN. The abrogation of this response by β-IFN-specific, neutralizing antiserum and inhibitors of RNA and protein synthesis strongly support the proposal that it is IFN itself that is responsible for the establishment of the refractory state and that it is not merely an effect of the binding of IFN to the cell surface.

β-Propiolactone-inactivated Sendai virus has been used throughout the experiments described here in order to restrict the effects of IFN to the early fusion or "fusion from without" (18) observed with this virus. Because this type of fusion does not require virus replication and results from an interaction between the viral glycoprotein and cell plasma membrane, it seemed highly probable that the observed effects of IFN were due to alterations in the outer cell membrane. This has been shown directly through the use of a fluorescent probe, DPH, that is capable of detecting molecular motions in the hydrocarbon core of the lipid bilayer and thus provide a measure of membrane fluidity. Treatment of human cells with homologous IFN yielded increased fluorescence polarization values that are consistent with a more rigid membrane structure. It is likely therefore that the inhibitory effect on Sendai virus-induced fusion observed in IFN-treated cells results from a decrease in the fluidity of the target cell plasma membrane.

The changes in membrane fluidity observed here in response to IFN treatment provide a rationale at the molecular level for many of the cellular effects observed after addition of IFN. Such changes could play a primary role in mediating the alterations in cell motility and growth described by others (1–3) because

many cellular processes appear to require information transfer across the plasma membrane. Alternatively, because treatment of both normal and transformed cells with IFN results in a significant reorganization of their cytoskeleton and extracellular matrix (2, 4), it is possible that such changes could, in turn, play a role in altering the fluidity of the cell plasma membrane. In addition to these cellular changes, the observed inhibition of murine retrovirus replication by IFN appears to involve a block in assembly at the stage of virus release from the cell (28–30). Such a block in IFN-treated cells would be consistent with a stiffening of the plasma membrane because, under conditions such that the membrane is less fluid, the process of budding might be expected to represent the rate-limiting step in viral replication. It will be of interest to determine whether the noninfectious particles released from IFN-treated cells contain a more rigid lipid envelope with less fusion activity than do virions released from untreated cells.

The experiments described here do not address the mechanisms that might be involved in changing the fluidity of the cell plasma membrane, nor is it known with what kinetics such a change takes place. The answers to such questions may be important in understanding the role of IFNs in cellular regulation.

Note Added in Proof. While this manuscript was being reviewed, Tomita and Kuwata (31) reported the inhibition of Sendai virus-induced cell fusion by IFN.

We thank Dixie W. Robinson for her help in preparation of this manuscript and Dr. P. M. Pitha for helpful discussions. This work was supported in part by Grant RO1 CA 27834-01 from the National Cancer Institute. E. H. is the recipient of Research Career Development Award K01 CA 00685-01 from the National Cancer Institute. S.C. is the recipient of Grant IN-66S from the American Cancer Society.

1. Brouty-Boye, D. & Zetter, B. R. (1980) *Science* **208**, 516–518.
2. Pfeffer, L. M., Wang, E. & Tamm, I. (1980) *J. Cell Biol.* **85**, 9–17.
3. Pfeffer, L. M., Murphy, J. S. & Tamm, I. (1979) *Exp. Cell Res.* **121**, 111–120.
4. Bourgeade, M. F., Rousset, S., Paulin, D. & Chany, C. (1981) *J. Interferon Res.* **1**, 323–332.
5. Chang, E. H., Jay, F. T. & Friedman, R. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1859–1863.
6. Lindahl, P., Gresser, I., Leary, P. & Tovey, M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1284–1287.
7. Vignaux, F. & Gresser, I. (1977) *J. Immunol.* **118**, 721–723.
8. Gresser, I. (1977) *Cell. Immunol.* **34**, 406–415.
9. Djeu, J. Y., Huang, K. Y., Holden, H.-T. & Herberman, R. B. (1980) *J. Exp. Med.* **151**, 781–789.
10. Chatterjee, S. & Hunter, E. (1980) *Virology* **104**, 487–490.
11. Chatterjee, S. & Hunter, E. (1979) *Virology* **95**, 421–433.
12. Chatterjee, S. & Hunter, E. (1980) *Virology* **107**, 100–108.
13. Friedman, R. M. (1977) *Bacteriol. Rev.* **41**, 543–567.
14. Baglioni, C. (1979) *Cell* **17**, 255–264.
15. Farrell, P. J., Sen, G. C., Dubois, M. F., Ratner, L., Slattery, E. & Lengyel, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5893–5897.
16. Sen, G. C., Lebleu, B., Brown, G. E., Kawakita, M., Slattery, E. & Lengyel, P. (1976) *Nature (London)* **264**, 370–373.
17. Roberts, W. K., Hovanessian, A., Brown, R. E., Clemens, M. J. & Kerr, I. M. (1976) *Nature (London)* **264**, 477–480.
18. Kohn, A. (1965) *Virology* **26**, 228–245.
19. Bratt, M. A. & Gallaher, W. R. (1969) *Proc. Natl. Acad. Sci. USA* **64**, 536–540.
20. Raj, N. B. K. & Pitha, P. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4918–4922.
21. Neurath, A. R., Strick, N., Raj, N. B. K. & Pitha, P. M. (1981) *J. Interferon Res.*, in press.
22. Taylor, J. (1964) *Biochem. Biophys. Res. Commun.* **14**, 447–451.
23. Sonnabend, J. A. & Friedman, R. M. (1973) in *Interferons and Interferon Inducers*, ed. Finter, N. (Elsevier North-Holland, New York), pp. 201–239.
24. Shinitzky, M. & Inbar, M. (1974) *J. Mol. Biol.* **85**, 603–615.
25. Cheung, H. C., Almira, E. C., Kansal, P. C. & Reddy, W. J. (1980) *Endocrine Res. Commun.* **7**, 145–156.
26. Rice-Evans, C., Bruckdorfer, K. R. & Dootson, G. (1978) *FEBS Lett.* **94**, 81–86.
27. Garland, F., Graves, D. E., Yielding, L. W. & Cheung, H. C. (1980) *Biochemistry* **19**, 3221–3226.
28. Chang, E. H., Mims, J. J., Triche, T. J. & Friedman, R. M. (1977) *J. Gen. Virol.* **34**, 363–367.
29. Billiau, A., Edy, V. G., DeClercq, E., Heremans, H. & DeSomer, P. (1975) *Int. J. Cancer* **15**, 947–953.
30. Pitha, P. M., Wivel, N. A., Fernie, B. F. & Harper, H. P. (1979) *J. Gen. Virol.* **42**, 467–480.
31. Tomita, Y. & Kuwata, T. (1981) *J. Gen. Virol.* **55**, 289–295.