Susceptibility to, and Production of Interferon by Rhinovirus (36638)

MILAN FIALA
(Introduced by L. B. Guze)

Research and the Medical Service, Veterans Administration Hospital (Wadsworth), Los Angeles, California 90073; Harbor General Hospital, Torrance, California 90509; and Department of Medicine, UCLA, Los Angeles, California 90024

The neutralizing antibody in nasal secretions has been shown to protect against infection by rhinovirus (1, 2); however, no effect of antibody on recovery from rhinovirus colds is apparent (1). Rhinovirus common cold is followed by a period of several weeks of nonspecific resistance to other respiratory virus infections, in which the action of an interferon mechanism was suggested (3). There is an increased interest in the treatment and prevention of rhinovirus infections by interferon inducers (4). The enhancement of plaque formation of some rhinoviruses by actinomycin D (5) which inhibits interferon production (6) and action (7) raises a possibility that rhinoviruses may cause autointerference. It is therefore important to determine the individual susceptibility of rhinoviruses to interferon and also their ability to induce its production. In this study we have developed quantitative methods to do so using rhinovirus type 2.

Materials and Methods. Cell cultures. HEK cells were obtained from the Flow Labs., Inc., Rockville, MD, as tube cultures containing approximately 1.5×10^5 cells. HEK cells were used for the assay and production of interferon within 7 days after planting. Tubes of Wl-38 strain of human embryonic lung were obtained from the same source as above.

M-Hela cells, which were propagated in this laboratory as previously described (5) were used for the plaque assay of vesicular stomatitis virus (VSV) and of rhinovirus.

Virus plaque assays. The plaque assay of rhinovirus in M-Hela cells has been described previously (5). For convenience, VSV was assayed by the same method except that plaques were visualized on the second instead of on the third day.

Viruses. Rhinovirus type 2 was obtained through the courtesy of Dr. D. Hamre, University of Chicago. This virus was purified from a single isolated plaque. Purified rhinovirus was further propagated in monolayers of M-Hela cells. VSV was obtained from Mrs. I. Zajac, Children's Hospital, Philadelphia. VSV stocks were prepared in M-Hela cells. Newcastle disease virus (NDV) was obtained from Dr. H. Blough, University of Pennsylvania, Philadelphia, and was kept at −90°.

Reference human interferon. Interferon induced by NDV in human leukocyte cultures was obtained through the courtesy of Dr. C. Chany, Center National de la Récherche Scientifique, Villejuif, France.

Preparation of interferon. HEK cells were infected at an initial multiplicity of rhinovirus of 0.3 to 30 PFU/cell or of NDV of 10 to 1000 PFU/cell. Eagle's minimum essential medium (Hanks' salt base) with 2% fetal calf serum and 5 m*M* bicarbonate (MEM FCa2) was used as maintenance medium. Infected cells were incubated for 48 hr at 34°. At the time of harvest, media were separated from cells, dialyzed overnight against 100 vol of glycine buffer (pH 2.2) and then against 100 vol of Hanks' balanced salt solution (BSS) with several changes.

Interferon assay. MEM FCa2 was used for dilution of interferon or as a control medium. HEK cells were incubated at 34–35° with 1:5 or higher dilution of an interferon specimen for 18 hr. Fluids were then discarded and the cells were washed once with BSS containing 0.2% fetal calf serum (BSS FCa2). VSV, at a multiplicity of 10 PFU/cell was adsorbed to the cells for 30 min

TABLE I. Susceptibility of Rhinovirus and VSV to Inhibition by Interferon in HEK Cells.

Virus	Interferon[a] dilution	Yield reduction (log)[b] Expt. 1	Expt. 2
VSV	1/5	1.40	2.69
	1/40	0.53	2.00
	1/160	0.40	—
Rhinovirus	1/5	0.94	1.04
	1/10	0.54	—
	1/40	0.14	0.45

[a] Reference human interferon.

[b] Log virus yield reduction in interferon-treated cultures compared to controls.

following which cells were washed, incubated in the dark for 24 hr and frozen at —90°. After thawing, VSV yields were assayed by the plaque method. Comparison of different specimens for interferon activity is based upon the log reduction of VSV yield.

Measurement of protein synthesis. To infected and control cultures containing 1×10^6 HeLa cells, 2 μCi/ml of valine-^{3}H was added, either continuously at 1 hr after infection or for 1 hr pulses at different times. At the end of labeling period, cells were washed, harvested into 0.5 ml of KOH and incubated for 10 min at room temperature. Then 0.5 ml of ice-cold 20% trichloroacetic acid (TCA) containing amino acids (twice the concentration present in MEM) was added. After 10 min of incubation the precipitated radioactivity was collected by filtration on glass-fiber discs, washed with 5% TCA and dried at 105° for 30 min. The radioactivity was then measured in a Tri-Carb scintillation spectrometer (Packard Instr. Co.) using Liquifluor (New Engl. Nuclear Corp.) as scintillation fluid.

Inactivation of virus by ultraviolet (UV) light. Rhinovirus stock (0.5 ml) was placed in a 60 mm tissue culture dish (pretreated by rinsing with MEM FCa10) and irradiated by UV light (germicidal lamp, G.E.) using a distance of 10 cm.

Results. Susceptibility of rhinovirus and VSV to interferon (Table I). Using the yield reduction method, rhinovirus and VSV were compared under the same conditions of batch and HEK cells, interferon dilutions and incubation conditions. Reference human interferon reduced VSV yields to a greater extent than rhinovirus yields, the difference between the two viruses being 0.5 log in one experiment and 1.6 log in another. Some inhibition of rhinovirus CPE by interferon was noted in HEK cells: 17 hr after infection, interferon-treated cells manifested about 25% degeneration while untreated cells showed 50% degeneration. In Wl-38 cells, however, rhinovirus CPE was not inhibited by interferon.

Stimulation of production of interferon by rhinovirus and NDV (Table II). Supernatant fluids collected from NDV- or rhinovirus-infected cell cultures and treated as described in Materials and Methods were tested for interferon activity against VSV. NDV induced appreciable interferon activity. Interferon activity, induced by rhinovirus at the highest multiplicity used (30 PFU/cell) was much lower than NDV-induced activity at comparable multiplicity. At low rhinovirus input multiplicity the interferon response was minimal.

Characterization of rhinovirus-induced interferon (Table III). Interferon prepared by acid treatment for 18 hr, as described, was distinct from infectious rhinovirus, as rhino-

TABLE II. Production of Interferon by HEK Cells Infected with Rhinovirus or NDV.

Virus	Expt.	m[a]	Interferon activity[b] 1/5	1/20	1/80
Rhinovirus	1	30	1.79	0.61	0.20
		0.3	0.81	0.41	ND
	2	30	0.20		
		30	0.25		
		30	0.25		
		3	0.25		
NDV	1	1000	3.04	2.79	2.14
	2	1000	4.14		
		30	3.84		
		10	2.70		

[a] Initial multiplicity of virus to HEK cells used for induction of interferon.

[b] Interferon activity measured by VSV yield reduction (log PFU) in HEK cells pretreated with interferon preparations at indicated dilutions.

TABLE III. Properties of Rhinovirus-Induced Interferon.

Interferon inducer	Treatment of interferon (1 hr)	Interferon activity[a]
Rhinovirus $m^b = 30$	Trypsin 0.025% at 37°	0.3
	Antiserum[c] 1/5 at 37°	1.0
	60°	0.0
	None	1.4

[a] Interferon activity measured at a dilution 1:5 as in Table II.
[b] See Table II.
[c] Human convalescent serum with neutralizing titer of 1/120.

virus infectivity is characteristically lost after 30 min at pH 3. Interfering substance was also distinct from viral proteins, as antiserum treatment did not abolish its activity. The activity of interferon was destroyed by trypsin and by temperature of 60°.

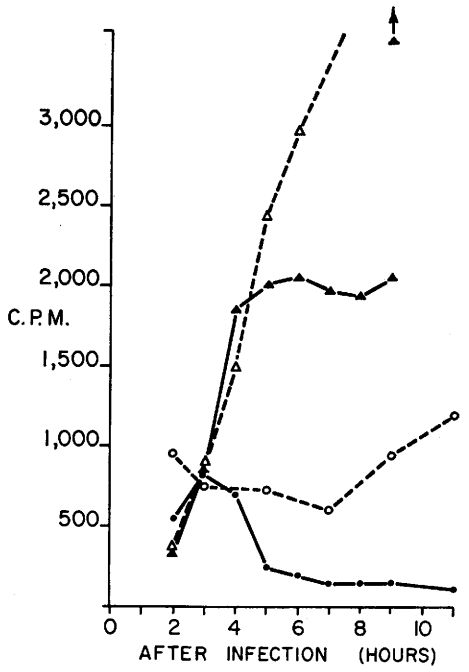

FIG. 1. Inhibition of host protein synthesis by infection with rhinovirus. Replicate cultures Hela cells were infected at initial multiplicity of 100 PFU/cell, washed after 1 hr and incubated at 34° in MEM FCa2. Cells were either labeled continuously with valine-^{3}H at the concentration of 2 μCi/ml added at 1 hr after infection [infected cells (▲), uninfected cells (△)] or pulse-labeled for 1 hr [infected cells (●), uninfected cells (○)]. The time on the horizontal axis indicates the end of labeling period. (cpm) TCA precipitable ^{3}H counts in each culture.

Inhibition of host-cell protein synthesis by rhinovirus (Fig. 1). Uninfected cultures incorporated between 600 and 1200 cpm of ^{3}H-valine/hr; total incorporation into protein proceeded in these cells linearly for 9 hr. In infected cells, the rate of incorporation dropped to less than 250 cmp/hr at 5 hr; total incorporation ceased to increase 5 hr after infection (Fig. 1).

Induction of interferon by inactivated rhinovirus. A possibility that inhibition of host cell protein synthesis by rhinovirus also inhibited interferon production was considered. When rhinovirus was inactivated by UV light, interferon activity decreased with increasing inactivation of virus (Table IV). The initial virus titer of 1.5×10^7 PFU was decreased by UV irradiation to 3×10^5 PFU after 1 min and to 4×10^3 PFU after 3 min.

Discussion. Rhinovirus susceptibility to interferon was assayed by the yield reduction method. This method was found to be a very sensitive assay for interferon with other viruses (8). Pretreatment with interferon for 18 hr before challenge and low bicarbonate concentration were chosen because some authors (1) show that these conditions increase the sensitivity of assay. In our study rhinovirus type 2 has been found susceptible to interferon, although less so than VSV. In a previous study rhinovirus type 2 was susceptible to interferon when assayed by a microplaque reduction method (10). Rhinovirus SD-1 was inhibited by interferon in calf trachea cultures (11). Rhinovirus type 13 was sensitive to inhibition by a synthetic

TABLE IV. Production of Interferon by UV-Inactivated Rhinovirus.

UV inactivation (min)	Residual infectivity[a] (%)	Interferon activity[b]
0	100	1.94
1	2	1.39
3	0.3	0.51

[a] Virus dose measured before inactivation was equal in each case to multiplicity of 30 PFU/cell.

[b] Interferon activity measured at dilution 1:5 as in Table II.

double-stranded RNA of polyriboinosinic and polyribocytidylic acids (poly I–poly C) at concentrations below those required to release interferon in cell culture (12). Although interferon administered intranasally exerted no protective effect against rhinovirus infection (13) prophylactic intranasal administration of poly I–poly C sgnificicantly ameliorated and in some human volunteers probably prevented experimental rhinovirus illness (4).

Stimulation of production of interferon has been tested in HEK cells which are good producers of interferon. Interferon production increased with increasing rhinovirus multiplicity, but even at the highest multiplicity used (30 PFU/cell) rhinovirus induced much less interferon compared to NDV at the same multiplicity. Virulent viruses such as Mengo virus and poliovirus which inhibit rapidly host cell RNA and protein synthesis (14, 15), do not induce interferon. It has been suggested that the lag period of interferon production is related to the lag period of virus multiplication (16). Rhinovirus-induced shutoff of host protein synthesis occurring at 5 hr precedes onset of virus replication by 1 hr, *i.e.*, in proper time to be able to inhibit interferon production. As UV inactivation of poliovirus type 1 abolished its cutoff of host protein synthesis (17) it can be surmised that rhinovirus-induced cutoff would also be prevented by UV inactivation of virus. It has been found, nevertheless, that UV-inactivated rhinovirus induced much less interferon than infectious rhinovirus. The lack of induction of interferon by rhinovirus, therefore, does not appear to be due to shutoff of host protein synthesis.

Tyrell (18) suggested that interferon might play a role in recovery from common cold. Most of the experimental studies reviewed by Baron (19) conclude that interferon plays a major role in recovery from established viral infections and also is one of the major determinants of nonspecific virus resistance. Andrewes (20) proposed a hypothesis suggesting an important role for interferon in the epidemiology of common cold viruses regarding their possible latency in the nose. Cate, Douglas and Couch (21) detected interferon activity in nasal wash of volunteers infected with rhinovirus type 15 and Coxsackie virus type A 21. The timing of interferon production was compatible with the interferon playing a role in recovery. However, as there was no enhancement of titer or of rapidity of production of interferon during the secondary virus infection the authors thought that interferon did not account for the temporary, nonspecific resistance against respiratory virus illness following a rhinovirus cold.

Our results indicate that, in cell culture, rhinovirus type 2 is sensitive to interferon, but not a good inducer of interferon, especially at low multiplicities or when inactivated by ultraviolet radiation. Further studies with other rhinovirus types are indicated. Synthetic inducers of interferon may become useful for stimulation of nonspecific interferon-mediated resistance as at least one rhinovirus is deficient in induction of interferon yet it is susceptible to its action.

Summary. Rhinovirus type 2 susceptibility to interferon and induction of interferon in human embryonic kidney cell cultures were compared to vesicular stomatitis (VSV) and Newcastle disease viruses, respectively. Rhinovirus was susceptible to interferon but less so than VSV. Rhinovirus was a very poor inducer of interferon even at the highest multiplicity tested (30 PFU/cell). Rhinovirus-induced shutoff of host protein synthesis probably did not account for poor stimulation of interferon as ultraviolet light-inactivated virus failed to induce interferon. Possible role of interferon in rhinovirus illness is discussed.

This study has been supported by grants from the Tuberculosis and Respiratory Association of California.

1. Cate, T. R., Rossen, R. D., Douglas, R. G., Jr., Butler, W. T., and Couch, R. B., Amer. J. Epidemiol. **84**, 352 (1966).

2. Perkins, T. C., Tucker, D. N., Knopf, H. L. S., Wenzel, R. P., Kapikian, A. Z., and Chanock, R. M., Amer. J. Epidemiol. **90**, 519 (1969).

3. Fleet, W. F., Couch, R. B., Cate, T. R., and Knight, V., Amer. J. Epidemiol. **8**, 185 (1965).

4. Hill, D. A., Baron, S., Perkins, J. C., Worthington, M., Van Kirk, J. E., Mills, J., Kapikian, A. Z., and Chanock, R. M., J. Amer. Med. Ass. **219**, 1179 (1972).

5. Fiala, M., Appl. Microbiol. **16**, 1445 (1968).

6. Heller, E., Virology **21**, 652 (1963).

7. Taylor, J., Biochem. Biophys. Res. Commun. **14**, 447 (1964).

8. Sreevalsan, T., and Lockart, R. Z., Jr., Virology **17**, 207 (1962).

9. De Mayer, E., and De Somer, P., Nature (London) **194**, 1252 (1962).

10. Sutton, R. N. P., and Tyrrell, D. A. J., Brit. J. Exp. Pathol. **42**, 99 (1961).

11. Smorodintsev, A. A., Brit. J. Exp. Pathol. **49**, 511 (1968).

12. Hill, D. A., Baron, S., and Chanock, R. M., Lancet **1**, 187 (1969).

13. Scientific Committee on Interferon, Lancet **1**, 505 (1965).

14. Zimmerman, E. F., Heeter, M., and Darnell, J. E., Virology **19**, 400 (1963).

15. Franklin, R. M., and Baltimore, D., Cold Spring Harbor Symp. Quant. Biol. **18**, 175 (1962).

16. Burke, D. C., *in* "Interferons" (N. B. Finter, ed.). North-Holland, Amsterdam (1966).

17. Penman, S., and Summers, D. F., Virology **27**, 614 (1965).

18. Tyrrell, D. A. J., "Rhinoviruses," Virol. Monogr. No. 2, Springer-Verlag, New York (1968).

19. Baron, S., *in* "Interferons" (N. B. Finter, ed.). North-Holland, Amsterdam (1966).

20. Andrewes, C. H., Science **146**, 1274 (1964).

21. Cate, T. R., Douglas, R. G., Jr., and Couch, R. B., Proc. Soc. Exp. Biol. Med. **131**, 631 (1969).

Received Apr. 24, 1972. P.S.E.B.M., 1972, Vol 140.