Interferon Induction by Rhinoviruses and Effect of Interferon on Rhinovirus Yields in Human Cell Lines[1] (37245)

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The rhinoviruses are the etiological agents responsible for a large proportion of acute upper respiratory illnesses (URI) in man known as the common cold (1, 2). Resistance to repeated illnesses by any one of the eighty-nine different serotypes (3) is due to type-specific antibody at the nasal membrane surfaces (4, 5). Recovery from a rhinovirus infection is followed by a transient period of nonspecific resistance to subsequent infection and establishment of URI by heterologous rhinoviruses; the period of nonspecific resistance can last for several weeks (6–8). Interferon may be one of the several factors responsible for nonspecific resistance (6–8). Previous studies have shown that interferon is present in nasal secretions and serum (9, 10) of human beings infected with different viruses. Production of interferon in hosts infected with viruses begins within hours of infection and continues for one to three weeks during acute virus infection *in vivo* (11).

The effect of interferon on the replication of human rhinoviruses has received little study. In one report, it was shown that replication of rhinovirus type 2 in monkey kidney cells was more sensitive to the antiviral effects of interferon than was replication of vaccinia virus, yellow fever virus, or an equine rhinovirus (12). In the present report, we examined the induction of interferon by rhinovirus serotypes 13, 14, and 20 in HeLa, KB, and L-132 cells and determined the effects of interferon on yields of these rhinoviruses in HeLa cell cultures.

Materials and Methods. Viruses. Rhinovirus serotypes 13 (RV13), 14 (RV14), and 20 (RV20) were obtained from V. V. Hamparian. Virus stocks were prepared in HeLa cells and assayed by a plaque method (13), except that Ionagar No. 2 (Colab Laboratories) was used to assay RV20 whereas Bacto-agar (Difco Laboratories) was used in the assay of RV13 and RV14. The propagation and assay of vesicular stomatitis virus, Indiana serotype (VSV), was previously described (14).

Cell cultures. HeLa, KB, and L-132 cells were grown as monolayer cultures in Blake bottles in Eagle's minimal essential medium containing 5% fetal calf serum. For experiments, the cells were removed by trypsinization and 1.6–1.8 $\times$ 10^6 cells were seeded to 60-mm plastic petri dishes and cultured for 16–18 hr at 34.5° in a CO_2 incubator prior to use.

Virus challenge and virus harvest. Growth medium or interferon-containing fluids were removed and the cell cultures were washed twice with phosphate-buffered saline (15) containing 0.1% bovine serum albumin, fraction V (PBSA). The virus inoculum contained in 0.2 ml was incubated with each cell culture for 2 hr at 34.5°. After removal of the virus inoculum, the cell cultures were washed three times with PBSA and 2 ml of MEM containing either 5% fetal calf serum (interferon-induction experiments) or 1% heat-inactivated fetal calf serum (effect of interferon on virus yield experiments) was added to each cell culture. At 24 hr post inoculation (pi), duplicate cell cultures were harvested for virus assay by scraping the cells into the fluids and the total was rapidly

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frozen and thawed three times to release virus.

Interferon. Interferon prepared in human foreskin cells using yellow fever virus, strain 17D, as an inducer (16) was a gift from W. Sibley. International human reference interferon was a gift from D. Lucas. J. Dean kindly provided us with human spleen cell interferon which was induced by phytohemagglutinin (17). Interferon content was measured by a plaque-assay technique using as end point that dilution which resulted in a 50% reduction in VSV plaques on HeLa cells. The interferon assay was carried out as previously described (18). Under our conditions, 5 ± 2 international reference human interferon units assayed as one unit.

Results. Experiments on induction of interferon by RV13, RV14, RV20, and NDV in HeLa cell cultures. HeLa cell cultures were challenged with each virus at two or three different multiplicities of infection (m.o.i.). After the 2-hr period for adsorption, the cell cultures were washed and incubated for 24 hr in MEM containing 5% fetal calf serum. The fluids were harvested and assayed for interferon content as described in "Materials and Methods." In both experiments shown in Table I, all three rhinoviruses failed to produce detectable levels of interferon. NDV induced 256 and 64 units/ml in these experiments. UV-irradiated viruses and heat-inactivated (56° for 20 min) viruses also failed to induce interferon in HeLa cells at a m.o.i. of 1 (RV13) or 4 and 50 (RV14 and RV20) plaque-forming units (pfu)/cell. As some strains of HeLa cells have been shown to be poor producers of interferon (19, 20), other types of cells were tested. KB and L-132 cells were challenged with the three rhinovirus serotypes at similar m.o.i. and the fluids from these cell cultures were assayed for interferon. All fluids from these cell lines did not contain detectable amounts (< 4 units/ml) of interferon. Several cell lines which do not produce interferon in response to certain viruses have been found to produce interferon if the cell cultures were incubated with interferon ("priming") prior to challenge with the viruses (21). This possibility was examined by incubating HeLa, KB, and L-132 cells with 10 units/ml of human spleen cell interferon for 18 hr prior to challenge with 0.5 pfu/cell of RV13 or with 0.5 and 50 pfu/cell of RV14 and RV20. All fluids from these cultures also contained less than 4 units/ml of interferon.

Attempt to induce resistance to VSV in HeLa cell cultures challenged with RV13, RV14, and RV20. Studies with polyinosinic acid–polycytidylic acid copolymer (pI:pC) have shown that antiviral resistance can be induced in cell cultures under conditions in which extracellular interferon could not be measured (22–24). In one study, this antiviral state was found to be mediated by interferon (25). The possibility existed that the three rhinovirus serotypes under study were inducing low levels of interferon that could not be detected by this assay system. We, therefore, assayed for induction of resistance by these rhinoviruses in HeLa cell cultures to plaque formation by VSV. HeLa cell cultures were challenged with UV-irradiated and heat-inactivated viruses. After a 2-hr period for adsorption, the cell cultures were washed and incubated for 18 hr at 34.5° in MEM containing 5% fetal calf

TABLE I. Attempts to Induce Interferon by RV13, RV14, and RV20 in HeLa Cell Cultures.

Virus	m.o.i. (pfu/cell)	Interferon yields (units/ml)	
		Expt. 1	Expt. 2
RV13	0.5, 5	<4	<4
RV14	0.5, 5, 50	<4	<4
RV20	0.5, 5, 50	<4	<4
NDV	0.5	ND[a]	64
	5.0	256	ND

[a] ND: not determined.

serum. The fluids were removed, the cell cultures were washed and challenged with approximately 100 pfu of VSV. After a 1-hr period for adsorption, the cell cultures were overlaid with a nutrient agar mixture and the monolayer cultures incubated for 2 days at 34.5°. VSV plaques were counted and the results are presented in Table II. The results clearly showed that none of the three inactivated rhinovirus serotypes induced any resistance to formation of VSV plaques on HeLa cell cultures. These results provided additional evidence that these three rhinoviruses do not induce interferon in HeLa cell cultures.

Effect of interferon on yields of RV13, RV14, RV20, and VSV in HeLa cell cultures. Although the rhinoviruses tested failed to induce detectable levels of interferon, it remained to be determined whether these viruses were susceptible to the antiviral action of interferon in HeLa cells. HeLa cell cultures were incubated with interferon for 16–18 hr, washed and challenged with 2 pfu (RV13) or 20 pfu (RV14, RV20, or VSV) per cell. After the 2-hr period for adsorption, the cell cultures were washed three times with PBSA and incubated with MEM for an additional 22-hr prior to harvest and assay for virus content. The results of two experiments are shown in Table III.

TABLE II. Lack of Induction of Resistance to VSV Plaque Formation in HeLa Cell Cultures Following Incubation with uv-inactivated and Heat-inactivated Rhinoviruses.

Virus	m.o.i. (pfu/cell)	VSV plaque no.[a]
None	—	100
UV-RV13	1	96
UV-RV14	5	105
UV-RV14	50	102
UV-RV20	5	91
UV-RV20	50	100
Heated RV13	1	93
Heated RV14	5	99
Heated RV14	50	96
Heated RV20	5	98
Heated RV20	50	99

[a] Average plaque count from 2 monolayer cultures.

RV13 was the least sensitive of all viruses tested to the action of interferon, very little inhibition in virus yields was found even at the highest interferon concentration tested. RV14 and VSV exhibited similar levels of sensitivity to interferon. The replication of RV20 was the most sensitive of all rhinoviruses tested to the action of interferon and even appeared to be more sensitive to the action of interferon than VSV. No significant ($< 10\%$) reduction in yields of RV13, RV14, or RV20 was observed in two experiments in which 25 units/ml of interferon were incorporated into the MEM that was added to cell cultures challenged with each virus serotype.

Discussion. Induction of interferon could not be demonstrated for any of the three rhinovirus serotypes tested on three different cell lines of human origin. NDV served as an effective inducer of interferon in the strain of HeLa cells used in this study, establishing that interferon could be induced by a virus in this cell line. Production of interferon by HeLa cells varies considerably with the strain under study (19, 20). KB cell cultures have been shown to respond to NDV as an inducer with 13-fold higher titers of interferon than HeLa cell cultures challenged under similar conditions (19). Inclusion of the more responsive KB cells and human embryonic lung cells (L-132) in the present study also failed to demonstrate interferon induction by the three rhinovirus serotypes. The present negative results are interpreted to mean that each of the rhinoviruses tested could not induce one or more steps in the process of induction of interferon in human cell lines of three different origins. "Priming," a function of interferon not related to its antiviral activity (21) can apparently compensate for some step(s) of the induction process that cannot be activated by some inducers. Indeed, it has been shown that L cell cultures which do not support replication of rhinovirus (Gauntt, unpublished data) can, if "primed," produce interferon in response to challenge with rhinovirus serotypes 1 and 2 but not in response to challenge by serotypes 14 or 51 (21). "Priming" also requires some response on the part of the cell line, as some cell lines

TABLE III. Effect of Interferon on Yields of RV13, RV14, RV20, and VSV in HeLa Cell Cultures.

Interferon[a] concentration (units/ml)	Virus yields (% control)[b]						
	Expt. 1			Expt. 2			
	RV13	RV14	RV20	RV13	RV14	RV20	VSV
400	ND	ND	ND	68	23	1	11
100	42	15	7	62	31	4	25
25	38	21	13	74	50	17	24
5	104	50	41	99	56	25	41
1	85	90	90	85	76	117	58
control	100	100	100	100	100	100	100

[a] Interferon used in Expt. 1 was produced by human foreskin cultures that had been challenged with yellow fever virus, strain 17D. In Expt. 2, the human international reference standard interferon was used.

[b] Virus yields in control cultures in Expt. 1 were 55, 333, and 112 pfu/cell for RV13, RV14, and RV20, respectively, and in Expt. 2, the yields were 886, 1135, 818, and 454 pfu/cell for RV13, RV14, RV20, and VSV, respectively.

can be "primed" more efficiently than others (26). "Priming" of the strain of HeLa cells used in this study did not render them more susceptible to induction of interferon by the three rhinovirus serotypes tested.

The three rhinovirus serotypes exhibited different sensitivities to the antiviral action of interferon, with the order of sensitivity being RV13 < RV14 < RV20. Hilleman and associates (27) studied the induction of resistance by pI:pC in human embryonic kidney cell cultures to several rhinovirus serotypes. They found a 7-fold difference in the minimum amount required to inhibit replication of these viruses, however, inhibition of replication of VSV by pI:pC required only 1/20th the amount required for inhibition of the most sensitive rhinovirus serotype. Resistance induced by pI:pC has been shown to be mediated via interferon (25). Other published data (12, 25) and the present study, therefore, suggest that interferon could play a role in amelioration of URI of rhinovirus etiology in man. An attempt to provide direct information to this point was made without success (28). Human volunteers given interferon by nasal instillation prior to challenge with rhinoviruses exhibited similar symptoms of URI illnesses as did placebo-treated control subjects (28). It was pointed out, however, that only low levels of interferon were used in that study.

Summary. Rhinovirus serotypes 13, 14, and 20 did not induce detectable levels of interferon in HeLa, KB, or L-132 cells following challenge with infectious, uv-inactivated, or heat-inactivated virus preparations. HeLa cell cultures incubated with interferon prior to challenge with the three rhinovirus serotypes also failed to produce interferon. No resistance to VSV plaque formation was induced in HeLa cell cultures previously challenged with uv- or heat-inactivated lysates of the three rhinovirus serotypes. Replication of the three rhinovirus serotypes in HeLa cell cultures was inhibited by the action of interferon with an order of sensitivity being RV13 < RV14 < RV20.

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Note added in proof: A recent report showed that rhinovirus serotype 2 induced very low levels of interferon in HeLa cells and replication of this virus was reduced by interferon in HeLa cells but less so than replication of VSV under similar circumstances. M. Fiala, Proc. Soc. Exp. Biol. Med. **140**, 1185 (1972).

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