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ANTI-INTERFERON ANTIBODY INCREASES RHINOVIRUS ISOLATION RATES FROM NASAL WASH SPECIMENS CONTAINING INTERFERON-ALPHA$_2$*

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Isolation of rhinoviruses from nasal secretions may be delayed or prevented by exogenously applied interferon which is carried over into nasal wash specimens. In studies of interferon-alpha$_2$ treated volunteers with experimental rhinovirus infection, we determined that incorporation of antiinterferon antibody into collection broth significantly increased isolation rates (84% increase) and the proportion of monolayers positive for CPE (155% increase), as compared to plain collection broth.

rhinovirus isolation; interferon-alpha$_2$; anti-interferon antibody

INTRODUCTION

In human fibroblasts or nasal epithelial cells a concentration-dependent antiviral effect can be obtained within minutes after addition of human leukocyte or fibroblast interferon (IFN) [1,2]. In epithelial cells this effect can persist for at least 72 h following removal of IFN [2]. Recently, Scott et al. [3] have reported that purified leukocyte IFN at concentrations of 100–1000 U/ml could delay or completely prevent rhinovirus type 9 isolation in HeLa cells from simulated nasal washes containing low virus concentrations.

We found that when human IFN-alpha$_2$ produced by recombinant DNA techniques (SCH30500, Schering Corporation, Bloomfield, New Jersey) was added to cell culture media in concentrations of 100–100 000 IU/ml 24 h prior to rhinovirus inoculation (10 $TCID_{50}$/monolayer), it could completely prevent CPE development in MRC-5 human embryonic lung fibroblast monolayers (HEM Research Incorporated, Rockville, Maryland). Subsequent in vitro experiments were conducted to investigate the utility of anti-IFN antibody and repetitive monolayer washing to reverse the IFN effect.

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* Written informed consent in a form approved by the University of Virginia Human Investigation Committee was obtained from all participants. Guidelines for human experimentation of the U.S. Department of Health and Human Services and the University of Virginia were followed in conducting this research.

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EXPERIMENTAL

Varying concentrations of IFN-alpha$_2$ and rhinovirus type 39 were added to lactated Ringer's solution (Cutter Laboratories, Inc., Berkeley, California), to simulate nasal wash specimens. These specimens were processed by mixing 3-ml volumes with 1 ml of 4-fold concentrated beef heart infusion broth (Difco Laboratories, Detroit, Michigan) containing 4% bovine serum albumin (Difco), 400 U/ml penicillin, 400 μg/ml kanamycin, and 4 μg/ml amphotericin B. 50 μl of sheep antibody to IFN-alpha$_2$, purified by ammonium sulfate precipitation (32.3 mg protein/ml), were added to selected collection broths. 50 μl of this antibody inhibited the antiviral activity of 10 000 IU (range 5000–20 000 IU) of IFN-alpha$_2$ in CPE inhibition assays using human foreskin fibroblasts exposed to encephalomyocarditis virus (W. Protzman, unpublished observation).

The effects of various methods of specimen processing on the development of rhinovirus CPE are shown in Table 1. The samples were allowed to absorb to the monolayers for 60 min after which monolayers were refed with maintenance medium. Depending

TABLE 1

Effects of adding anti-IFN antibody and of repetitive monolayer washing on the development of rhinovirus CPE in human embryonic lung fibroblast monolayers inoculated with simulated nasal wash specimens containing IFN

Virus inoculum ($TCID_{50}$) per monolayer	Interferon concentration (IU/ml)	Type of specimen processing[a]			
		Refeed	Antibody in broth + refeed	PBS wash 3X + refeed	Antibody in broth + PBS wash 3X + refeed
15	0	12/12 (3)[b]	–[c]	5/5 (4)	–
	1 000	4/5 (4)	–	3/4 (4)	4/4 (3)
	10 000	6/6 (5)	6/6 (3)	6/6 (4)	6/6 (3)
1.4	0	8/12 (3)	–	3/5 (4)	–
	1 000	4/6 (6)	4/6 (4)	4/6 (5)	2/6 (–)
	10 000	4/6 (6)	2/6 (–)	2/6 (–)	6/6 (4)
	100 000	0/6 (–)	0/6 (–)	1/6 (–)	3/6 (7)

[a] Simulated nasal washes were mixed (3:1, v/v) with plain collection broth or broth containing anti-IFN antibody. Aliquots of this mixture were adsorbed on fibroblast monolayers for 60 min at 33°C. After removal of the inoculum, monolayers were either refed with maintenance medium or washed three times with phosphate-buffered saline, and then refed.

[b] No. monolayers positive for rhinovirus CPE/total no. exposed (median day to positivity). Monolayers were observed daily for 7 days. A monolayer was considered to be positive, if typical, focal CPE that progressed to at least 50% involvement of the monolayer was present. Uninfected monolayers exposed to interferon 100 000 U/ml did not show CPE.

[c] Abbreviations: – = not determined; PBS = phosphate-buffered saline.

on the concentration of IFN and on the size of the virus inoculum in the simulated nasal wash, either a delay in appearance of CPE or complete prevention of CPE was found. The addition of anti-IFN antibody to the collection broth and repetitive washing of the monolayers after adsorption of the virus inoculum reversed this effect. When very high IFN concentrations were present in simulated specimens (e.g. 100 000 IU/ml), the combined use of both processing techniques gave the highest rate of positive culture results, although the median time to positivity was still 4 days longer than when no IFN was included in the specimens. These observations suggested that routine isolation procedures may underestimate the frequency of virus shedding in individuals given high dosages of intranasal IFN.

Since the inhibitory effect of IFN was partially reversed by repetitive washing of the monolayers and by addition of anti-IFN antibody to the collection broth, we used these methods to process samples collected from volunteers experimentally infected with rhinovirus type 39. Two placebo-controlled, double-blind volunteer studies were conducted. In the first study, volunteers (n = 12) were given IFN-alpha$_2$ ($\sim 11.4 \times 10^6$ IU/dose) by intranasal drops four times per day for 4 days beginning 1 day before virus challenge (daily dose $\cong 46 \times 10^6$ IU). In the second trial, volunteers (n = 14) were given IFN-alpha$_2$ (31×10^6 IU/dose) once daily for 5 days beginning 2 days before virus challenge (daily dose $\cong 31 \times 10^6$ IU).

Nasal wash specimens were collected from all participants each morning on the first through fifth days after virus challenge. These samples were collected at 8–9 h after preceding the IFN dose in the first study and at 22–24 h in the second. All specimen collections and processing were performed by individuals unaware of the treatment status of the volunteer. 2–3-ml aliquots of lactated Ringer's solution were instilled in each nostril and the effluents were collected. This process was repeated once or twice, until a minimum volume of 7 ml per participant was obtained. Washes were mixed by repeated aspiration through a 19 gauge needle, and 3-ml aliquots were dispensed into glass vials containing 1 ml of collection broth with or without anti-IFN antibody (see above). Both samples were held on wet ice, and 0.4-ml aliquots were inoculated within 2 h of collection onto three fibroblast monolayers (total volume 1.2 ml per sample). MRC-5 fibroblasts either at passage levels 27-28 (MA Bioproducts, Walkersville, Maryland) or at passage levels 21-26 (HEM Research) were used. Only monolayers from a single lot of cell cultures were used on a given day of specimen processing. Maintenance media was 1:1 mixture of Eagle's minimal essential medium and medium 199 supplemented with 2% newborn bovine (MA Bioproducts) or fetal bovine serum (HEM Research), 100 U/ml penicillin, 100 μg/ml kanamycin, and 1.0 μg/ml amphotericin B. Samples were adsorbed for 60 min at 33°C, and the monolayers were washed three times with phosphate-buffered saline (pH 7.2–7.4) and then refed with fresh maintenance medium. At least one isolate from each volunteer was confirmed to be rhinovirus type 39 by standard neutralization testing.

Table 2 lists the proportions of all virus-containing specimens that were positive when collection broth with or without anti-IFN antibody was used for processing nasal

TABLE 2

Effect of adding anti-IFN antibody to collection broth for the recovery of rhinovirus from nasal wash specimens of experimentally infected volunteers

Treatment given volunteers (days of specimen collection)	Antibody in collection broth[a]	No. specimens positive/ total no. of specimens positive with either method of processing (%)	No. monolayers positive/ total no. of monolayers inoculated with positive specimens (%)
Placebo (days 1–5 after virus exposure)	no	67/78 (85.9)	157/231 (68.0)
	yes	71/78 (91.0)	155/234 (66.3)
Interferon (days 1–5 after virus exposure)	no	12/17 (70.6)	23/51 (45.1)[b]
	yes	15/17 (88.2)	34/51 (66.7)[c]
Interferon (days 1–4 only)	no	6/11 (54.5)[c]	9/33 (27.3)[d]
	yes	11/11 (100)[c]	23/33 (69.7)[d]

[a] 50 μl of sheep antibody (32.2 mg protein/ml) to IFN-alpha$_2$, sufficient to inhibit 10 000 IU of interferon, was added to 1 ml of collection broth. 3 ml of nasal wash were mixed with 1 ml of 4-fold concentrated collection broth with or without anti-IFN antibody. See text for details of specimen processing.

[b] $P = 0.046$, Fisher exact test, two-tailed.

[c] $P = 0.035$.

[d] $P = 0.001$.

washes. When all postchallenge days were combined for analysis, a trend toward an increased frequency of virus isolation was evident in IFN-alpha$_2$ recipients whose specimens were processed with broth containing antibody. Compared to plain broth, the antibody-containing broth was associated with a 24.9% higher isolation rate. When the fifth postchallenge day, in which IFN was not present in nasal wash specimens, was excluded from analysis, the virus isolation rate was significantly higher with antibody-containing broth than with plain broth. Relative to plain broth, the addition of antibody increased the rate of rhinovirus isolation by 83.5% on these days. Similarly, the proportion of fibroblast monolayers inoculated with positive specimens that were positive for rhinovirus CPE was significantly higher, when these samples were processed with broth containing antibody. For IFN recipients, the proportion of CPE positive/total monolayers increased by 47.9% for all postchallenge days and 155.3% for the first through fourth days, when antibody was added to the collection broth.

No effect of antibody addition on isolation rate was found in placebo recipients. Since the collecting and processing of samples were carried out without knowledge of treatment status, this finding confirms that the increased isolation rate in IFN recipients was specifically related to the use of anti-IFN antibody. In contrast to this finding, Scott et al. [3] noted increased rhinovirus isolation rates in both IFN and placebo recipients,

whose initially negative specimens were reprocessed with the addition of anti-IFN antibody. However, these authors did not indicate whether the reisolation efforts were controlled by parallel reprocessing of samples without antibody addition. In our volunteer studies we did not separately test the utility of repetitively washing monolayers exposed to IFN-containing samples, but did find that washing alone was not sufficient to reverse the CPE inhibitory effect of IFN.

Further studies of intranasally applied IFN should consider incorporation of anti-IFN antibody in collection broth to avoid underestimating the frequency of virus shedding.

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