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Effect of Human Nasal Secretions on the Antiviral Activity of Human Fibroblast and Leukocyte Interferon[1] (39448)

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Since the discovery of interferon (IF), investigators have sought to utilize this antiviral agent clinically. One potential use of IF would be in treating or preventing viral respiratory tract infections, since it has been implicated in recovery from such infections (3). In addition, the antigenic diversity of viral pathogens complicates control by conventional vaccination approaches.

Most clinical trials with intranasally applied IF have failed to show efficacy (8). However, Merigan *et al.* recently demonstrated that clinical efficacy against a rhinovirus challenge was achieved when large quantities of human leukocyte IF were administered (10).

Because several reports have indicated that various body fluids are capable of inhibiting IF (4, 5, 14), we examined human nasal secretions for the presence of an inhibitor that could account for the apparent large dose requirement necessary for clinical efficacy. The effect of human nasal secretions on the antiviral activity of both human fibroblast and leukocyte IF was investigated because of the described antigenic and biochemical differences between leukocyte and fibroblast IF (1, 15).

Materials and methods. Nasal secretions. Nasal secretions from healthy volunteers were collected and concentrated 10-fold by a previously described procedure (13). Total protein concentrations were determined by the technique of Lowry *et al.* (9).

Interferon preparations. Both human fibroblast and leukocyte IF were provided by the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases. Fibroblast IF was prepared by Havell and Vilček (7) using human foreskin fibroblasts and polyriboinosinic:polyribocytidylic acid as the inducer. Leukocyte IF was prepared from buffy coat leukocytes stimulated with Sendai virus and purified according to the technique of Cantell (2).

Cell cultures. Human foreskin fibroblasts (cell strain HR-202) from HEM Research Inc. (Rockville, Md.) were used to assay IF. Mouse L-cells were used for plaque assays of vesicular stomatitis virus (VSV). Both cell lines were propagated in Eagle's minimum essential medium with Earle's salts (MEM) purchased from Grand Island Biological Co. (Grand Island, N.Y.). MEM was supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 μg/ml), and *l*-glutamine (10 m*M*). Cells were cultured at 37° in an atmosphere of 5% CO_2 and 95% air.

Interferon assay. A yield reduction assay was used to measure IF activity. Samples for assay were diluted in MEM containing 5% FBS and 1-ml aliquots were added in duplicate to HR-202 cell monolayers (1.0×10^6 cells) in 10 × 35-mm plastic petri dishes (Lux Scientific Corp.). After incubation at 37° for 18–24 hr, the medium was removed and the cells were washed once with phosphate-buffered saline (PBS) deficient in calcium and magnesium ions (6). The HR-202 monolayers were then challenged with VSV at a multiplicity of infection of at least 10. After adsorption for 1 hr at 37°, the inoculum was removed and the cells were washed four times with PBS. The monolayers were then refed with 2 ml of MEM containing 2% FBS and incubated at 37°. After 24 hr, the fluid was collected and centrifuged at 1400*g* for 10 min, and the supernatant fluid was frozen at −70° until assayed for VSV.

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Virus yields were determined by plaque assay in L-cells. One unit of IF was defined as that dilution of the original sample which reduced the VSV yield by 50% compared to controls. In this system, 5 units of IF were equivalent to 1 unit of the human IF research standard 69/19 from the Medical Research Council (Mill Hill, London, England). An internal standard was included with most assays and the titer did not vary more than twofold.

Results. Effect of nasal secretions on varying doses of human fibroblast IF. To determine the effect of nasal secretions on fibroblast IF, 0.15 ml of concentrated nasal secretions from 10 individuals was each mixed well with an equal volume of MEM (without serum) containing 75, 750, or 7500 units of fibroblast IF. As controls, the same quantities of IF were mixed with MEM. All mixtures were incubated for 4 hr at 34°, to simulate the temperature of the upper respiratory tract. The volume of each sample was then increased to 3.0 ml to yield final IF concentrations of 25, 250, or 2500 units/ml, and 1.0-ml aliquots were assayed for IF activity. Twenty-five units of fibroblast IF incubated with MEM reduced the virus yield from 7.0×10^8 plaque forming units (PFU)/ml (control) to 1.1×10^6 PFU/ml (Fig. 1). However, when the same amount of IF was incubated with nasal secretions from 10 individuals, an equivalent reduction in virus yield did not occur. Yields of VSV varied from 6.6×10^6 to 9.4×10^7 PFU/ml, representing a 6- to 85-fold increase in virus yield and a loss of 38 to 91% of the IF activity. The mean virus yield from cells treated with IF and nasal secretions was increased by more than 2 standard deviations over the IF control virus yield. The loss of IF activity was attributable to interaction with nasal secretions, since nasal secretions alone in this assay system increased the virus yield by an average of only 1.7-fold. The inhibition of IF activity was overcome when the same nasal secretions were incubated with 250 or 2500 units of fibroblast IF (Fig. 1).

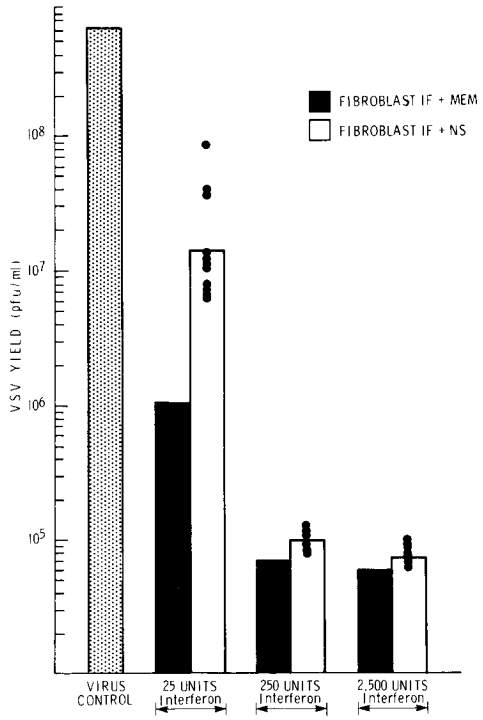

FIG. 1. Effect of human nasal secretions on the antiviral activity of human fibroblast interferon (IF). The results following incubation of 10 individual nasal secretions (NS) with 25, 250, or 2500 units of IF are represented by dots; geometric means are represented by open bars.

Comparison of the effect of nasal secretions on human fibroblast and leukocyte IF. Six nasal secretion specimens were incubated with approximately 25 units of human fibroblast or leukocyte IF (Fig. 2). Three of the nasal secretions incubated with fibroblast IF did not significantly increase virus yields and therefore did not inhibit IF. However, three other nasal secretions did increase virus yields by 13- to 18-fold. In this experiment the mean increase in virus yield was also 2 standard deviations greater than the IF control. In contrast to the results obtained with fibroblast IF, five of these six nasal secretions did not significantly enhance virus yields when incubated with leukocyte IF.

Similar experiments have been repeated with a total of 28 nasal secretions from different individuals. Using a fourfold increase in virus yield as evidence of inhibition of IF activity, 57.1% of the nasal secretions tested inhibited fibroblast IF, whereas only 10.7% of the same nasal secretions inhibited leukocyte IF (Table I). Moreover, a 4.6-fold increase in virus yield was the greatest increase noted with leukocyte IF,

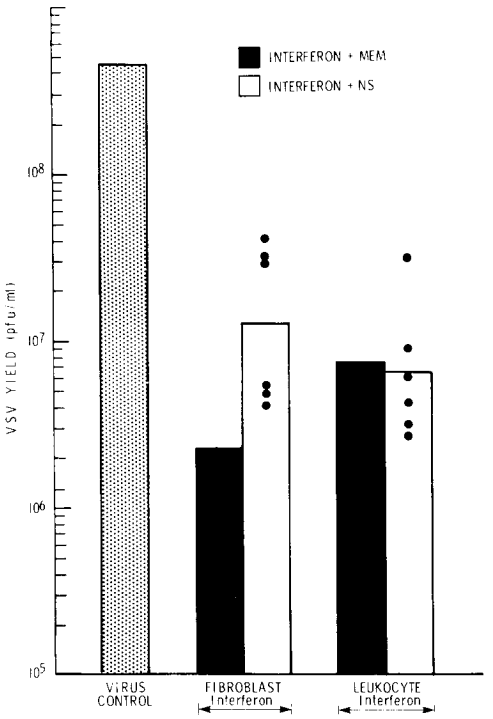

FIG. 2. Effect of human nasal secretions on the antiviral activity of human fibroblast and leukocyte interferon. The results following incubation of six individual nasal secretions (NS) with approximately 25 units of fibroblast or leukocyte interferon are represented by dots; geometric means are represented by open bars.

whereas nasal secretions showing inhibitory activity toward fibroblast IF caused an average virus yield increase of 15.7-fold.

To rule out the possibility that leukocyte IF was simply less sensitive than fibroblast IF to the inhibitor in nasal secretions, increasing amounts of nasal secretions were incubated with fibroblast or leukocyte IF. To conduct this and subsequent experiments, a nasal secretion pool was prepared by combining the secretions obtained from 25 volunteers.

Fifty units of fibroblast or leukocyte IF in a constant volume (0.15 ml) were incubated with 0.075, 0.15, 0.3, or 0.6 ml of the nasal secretion pool. This gave a ratio (volume of nasal secretion to volume of IF) of 0.5:1, 1:1, 2:1, or 4:1, respectively. Neither IF preparation was noticeably affected by incubation with increasing volumes of MEM (Fig. 3). In addition, the increase in nasal secretion volume did not result in a concomitant increase in virus production in the absence of IF. However, when increasing volumes of the nasal secretion pool were incubated with fibroblast IF, a linear increase in virus yield (decrease in IF activity) was noted; a 4:1 volume ratio of nasal secretion to IF resulted in total inhibition of 50 units of fibroblast IF. In contrast, leukocyte IF was not inhibited by increasing amounts of nasal secretions.

Preliminary characterization of the IF inhibitor(s) in nasal secretions. In three sepa-

TABLE 1. NUMBER OF NASAL SECRETIONS (NS) SHOWING INHIBITION OF INTERFERON ACTIVITY.

Experiment	Number of NS	Number of nasal secretions showing inhibitory activity against[a]	
		Fibroblast IF	Leukocyte IF
1	6	6	0
2	6	3	1
3	7	3	1
4	7	3	1
5	2	1	0
Totals	28	16 (57.1%)	3 (10.7%)

[a] Defined as an increase in virus yield of fourfold or greater compared to the interferon control.

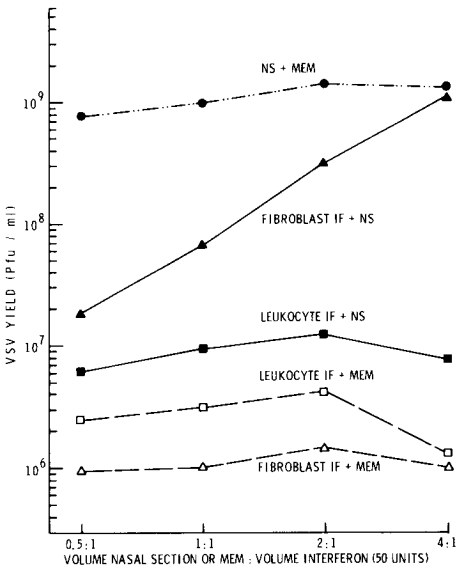

FIG. 3. Effect of increasing volumes of nasal secretions (NS) on the antiviral activity of human fibroblast and leukocyte interferon (IF).

rate experiments, the inhibitory activity was greatest in those nasal secretions with the highest total protein determinations. Initial attempts to identify this inhibitor in nasal secretions were carried out by incubating fibroblast IF with pure preparations of three of the major proteins [i.e., albumin, lysozyme and IgA (11, 12)] found in nasal secretions. A range of physiological concentrations of albumin (0.1, 1.0, and 10.0 mg/ml, Behring Diagnostics), lysozyme (0.1, 1.0, and 10.0 mg/ml, Miles Laboratories), and 7S IgA (0.15, 1.5, and 15.0 mg/ml, Behring Diagnostics) did not possess inhibitory activity. Therefore, to characterize the properties of the inhibitor in nasal secretions, portions of the nasal secretion pool were subjected to the following treatments and then tested for inhibitory activity. Nasal secretions were dialyzed for 3 hr against three changes of 400 ml of PBS. The inhibitor was nondialyzable. Heating at 56° for 1 hr did not deplete nasal secretions of inhibitory activity. Portions of the nasal secretion pool were incubated at 37° for 1 hr with trypsin (5 mg/ml, Flow Laboratories, Inc.). The reaction was stopped by the addition of FBS at a final concentration of 33%. The inhibitor was not destroyed and the presence of the increased serum concentration did not appear to influence the IF assay.

The nasal secretion pool was also mixed continuously with 10 vol of ether for 5 min. The aqueous phase was dialyzed against PBS and tested for inhibitory activity. Following this treatment, the nasal secretion pool did not inhibit IF. Portions of the nasal secretion pool were subjected to 100,000*g* for 2 hr in a Beckman Model L3-50 ultracentrifuge using a Type 40 rotor. After centrifugation, the top two-thirds of the fluid was recentrifuged at 100,000*g* for 2 hr. The inhibitory activity was found in the upper two-thirds of the fluid following the second centrifugation.

Discussion. We have examined human nasal secretions for the presence of an inhibitor of human fibroblast or leukocyte IF. The presence of such an inhibitor might explain the need for such large doses of leukocyte IF to show *in vivo* efficacy (10). The antiviral activity of small quantities of human fibroblast IF was partially inhibited when mixed with human nasal secretions. This inhibition was overcome with increased concentrations of fibroblast IF. Human leukocyte IF, however, was not inhibited by human nasal secretions.

In view of the described antigenic (1) and biochemical (15) differences between fibroblast and leukocyte IF, it is not surprising that biological differences, such as sensitivity to an inhibitor, do exist. Other body fluids which have been shown to inhibit fibroblast IF are serum (4, 14), saliva, cerebrospinal fluid, bile, an extract of feces (4), and urine (5). The same body fluids were not tested against leukocyte IF. However, an inhibitor of leukocyte IF in serum has been demonstrated (Stanton and May, personal communication).

Nasal secretions are a complex mixture of substances which include at least 12 of the proteins found in plasma (12) as well as six components which cannot be detected in plasma (11). Although our results do not allow a definitive identification of the inhibitor, three of the major proteins intrinsic to nasal secretions (i.e., albumin, lysozyme, and 7S IgA) do not appear to be responsible. The characteristics of the inhibitor found in nasal secretions resemble those of an inhibitor found in fetal bovine and human serum (14). These investigators suggested that the inhibitor they described might be associated with a lipoprotein. Our results concerning the properties of the inhibitor described in this report are not inconsistent with a similar interpretation.

Although an inhibitor of human fibroblast IF was found in the present study, it does not explain the results obtained in clinical trials with human leukocyte IF, since no inactivation or inhibition of leukocyte IF by human nasal secretions could be demonstrated. The inhibitor of fibroblast IF, however, could be of significance if this preparation were to be considered for clinical use.

Summary. Many normal human nasal secretions contain an inhibitor of human fibroblast IF. This inhibitor had no effect on human leukocyte IF. The amount of inhibition of fibroblast IF increased with increasing quantities of nasal secretions. Also, the inhibition could be overcome with increasing concentrations of IF.

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