

Rhinovirus infection induces mucus hypersecretion

ATSUSHI YUTA,¹ WILLIAM J. DOYLE,² ETHAN GAUMOND,¹ MUSHTAQ ALI,¹ LAWRENCE TAMARKIN,³ JAMES N. BARANIUK,¹ MATTHEW VAN DEUSEN,⁴ SHELDON COHEN,⁵ AND DAVID P. SKONER⁴

¹*Division of Rheumatology, Immunology, and Allergy, Georgetown University Medical Center, Washington, District of Columbia 20007-2197; Departments of*

²*Otolaryngology and*
⁴*Pediatrics, Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine, and*
⁵*Department of Psychology, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213; and*
³*CYTImmune Science, College Park, Maryland 20770*

Yuta, Atsushi, William J. Doyle, Ethan Gaumond, Mushtaq Ali, Lawrence Tamarkin, James N. Baraniuk, Matthew Van Deusen, Sheldon Cohen, and David P. Skoner. Rhinovirus infection induces mucus hypersecretion. *Am. J. Physiol.* 274 (*Lung Cell. Mol. Physiol.* 18): L1017–L1023, 1998.—Rhinorrhea is a prominent symptom of the common cold. Although increases in vascular permeability and serous cell secretion have been demonstrated in human nasal mucus during active rhinovirus infections, changes in mucin constituents have not been quantified. Nonallergic ($n = 48$) and asymptomatic allergic rhinitis ($n = 32$) subjects were inoculated with rhinovirus type *hanks* before the spring allergy season. Nasal lavages were performed before inoculation (*day 0*), then daily for 5 days afterward. The subjects were divided into infected and noninfected groups on the basis of evidence of successful rhinovirus infection (nasal shedding of virus or fourfold increases in specific serum antibodies). Concentrations of interleukin (IL)-8, markers of vascular leak (IgG), seromucous cells (lysozyme), and mucoglycoprotein exocytosis [7F10-immunoreactive mucin (7F10-irm) and Alcian blue staining of acidic mucoglycoproteins] were measured in lavage fluids. The infected subgroup had maximal increases in nasal lavage fluid concentrations of IL-8 (sevenfold), IgG (fourfold), total protein (twofold), and gel-phase 7F10-irm (twofold) on *day 3*. There were no differences between infected allergic and nonallergic subjects. IL-8 and gel-phase 7F10-irm were significantly higher in infected than in noninfected subjects. In addition to promoting plasma exudation, rhinovirus *hanks* infection increases IL-8 and gel-phase mucin secretion. These processes may contribute to a progression from watery rhinorrhea to mucoid discharge, with mild neutrophilic infiltration during the common cold.

nasal secretion; interleukin-8; vascular permeability; glandular exocytosis; mucin

RHINOVIRUS INFECTION is the most common cause of colds in adults and children (11). Rhinorrhea is a major symptom. The macromolecules in nasal secretions originate from four sources: 1) vascular permeability via postcapillary venules and fenestrated capillaries, 2) exocytosis from submucosal gland seromucous cells, 3) exocytosis from epithelial goblet and submucosal gland mucous cells, and 4) infiltrating leukocytes (20). Rhinovirus type 39 (RV-39) infection leads to an increase in vascular permeability that coincides with maximal

symptom complaints followed by serous cell exocytosis of secretory IgA (sIgA) and lysozyme and a mild neutrophilic infiltrate (17, 23). Vascular permeability may flood the mucosa with virus-specific IgG, whereas mucosal stimulation may lead to local production of virus-specific IgA. This IgA is transported via submucosal gland serous cells as sIgA (17). In addition, serous cells contribute lysozyme, lactoferrin, hyaluronan, and "neutral mucins" (2, 25). Epithelial goblet and submucosal gland mucous cells secrete sulfated, sialylated "acid" mucins, but changes in secretion of these mucoglycoprotein (MGP) components are poorly appreciated (20).

Quantitative changes in these constituents of nasal mucus may be of pathophysiological importance because they may alter local mucosal host defenses (20). Increases in the volume of nasal secretions combined with virus-induced impairments in mucociliary clearance may lead to local pooling of virus-laden secretions in the nasopharynx (6, 32). The combination of mucins plus plasma fibrinogen may generate thick mucus "clots." A sufficient volume of these fluids at the sinus ostia and eustachian tube orifices may occlude fluid flow and lead to the retention of secretions in the sinuses and middle ear. This is consistent with the appearance of mucosal thickening and air-fluid levels evident on sinus computerized tomography scans during rhinovirus infections (3, 13). Colonization of this "culture media" within the now closed spaces of the middle ear and paranasal sinuses may then lead to purulent bacterial infections (3, 13, 23, 30). Neutrophil infiltration could occur in response to bacterial products, interleukin (IL)-8 released from epithelial cells or other sources, or other chemoattractant signals (15, 16, 23).

In this report, the kinetics of secretion production during a rhinovirus cold was explored with experimental exposure of adult volunteers to rhinovirus type *hanks* (RV-h). Nasal lavage fluids were collected before and for 5 days after inoculation so that serial changes in mucus constituents could be measured. IL-8 was measured as a marker of the inflammatory response. IgG was a marker of vascular permeability. Lysozyme identified submucosal gland serous cell exocytosis. MGPs were assessed by assaying Alcian blue-staining MGP (ABSM) and high-molecular-weight 7F10-immunoreactive mucin (7F10-irm). For data analysis, subjects were divided into infected and noninfected groups. The subjects were also stratified by allergy status to determine whether there were differences in mucus

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

production between allergic and nonallergic subjects (18, 27).

MATERIALS AND METHODS

Protocol

Subjects. Subjects were recruited by newspaper advertisement from the population and surrounding community of the University of Pittsburgh (PA). Potential subjects were screened by history for previous nasal or otologic disease, were given a general physical examination, had a urinalysis, and had phlebotomy for assays of markers of hepatic and renal function and for measurement of serum antibodies to human immunodeficiency virus (HIV) and RV-h with standard methods (5, 27). Subjects were excluded if they presented with findings of, or a history suggestive of, systemic illness or recent upper respiratory tract infection, if they had marked elevations in the assayed parameters indicative of hepatic or renal impairment, if they required prescription medication for any condition other than birth control, or if they had antibodies to HIV. The study was approved by the Human Rights Committee at the University of Pittsburgh, and all subjects provided written informed consent for HIV screening and study participation.

The study population for this report consisted of 80 healthy, asymptomatic adult volunteers (age range 18–52 yr) who were studied in March 1995. At the time of study, the subjects and investigators were blinded to the prechallenge-specific serum RV-h antibody levels and to the allergy status of the subjects.

Rhinovirus inoculation protocol. Eligible subjects were cloistered on *day 0* in separate rooms of a local hotel for a 6-day period (study *days 0–5*). On each day of cloister, symptoms were scored by the subjects, signs were evaluated by a physician, temperatures and vital signs were recorded, and a nasal lavage was performed (13).

Twenty-four hours after admission to cloister (end of study *day 0*), all subjects were intranasally inoculated with ~300 units of a 50% tissue culture infection dose of a safety-tested clinical isolate of RV-h (supplied by Dr. Jack Gwaltney, Charlottesville, VA) delivered as coarse drops in two doses within a 15-min period (5, 12). At the end of study *day 5*, the subjects were dismissed from the cloister, but they returned to the laboratory on *days 19–21* for phlebotomy to assess convalescent antibody titers and for the evaluation of allergy status.

Symptoms. Subjects scored eight symptoms (5–7, 27). The sum of sneezing, congestion, and rhinorrhea replies was termed the nasal score. The sum of cough and sore throat results was the throat score. The general symptoms score was the sum of headache, malaise, and chills complaints.

Nasal lavage. Daily nasal lavages were performed each morning with previously described methods (7, 17). Briefly, 5 ml of saline were instilled into each of the nares while the subject hyperextended his/her neck to a 60° angle and closed his/her velopharyngeal port. After 5–10 s, the fluid was forcibly expelled into a collection vessel. The recovered fluid averaged ~6 ml and was subdivided into aliquots for virus culture and chemical assays and stored at –70°C. The relative contributions of saline and nasal secretions to the recovered lavage fluid volume could not be defined in this protocol but were assumed to be equivalent in all lavages.

Allergy diagnosis. An allergic diagnosis was made on the final day of the study based on a positive history (with appropriate seasonal illness) and positive prick skin tests (>10-mm flare) to ragweed, grass, or tree allergens and/or serum IgE titer (>100 IU/ml) by fluoroallergosorbent assay.

Rhinovirus titers. Paired specific serum antibody titers to RV-h were measured before and on *days 19–21* after rhinovirus exposure with methods previously described (14). Daily nasal lavage samples were cultured for RV-h virus with previously described methods (21). Subjects were defined as infected if virus was recovered on any day after experimental exposure or if they had a fourfold rise in RV-h-specific serum antibody titer.

Assays

Gel and sol phases. For the assay of MGP components, aliquots of lavage fluid were thawed at 4°C, and 500- μ l samples were separated into the sol and gel phases by centrifugation at 10,000 *g* for 10 min at 4°C. The supernatant was separated and used for sol-phase component assays, and the pellet was resuspended with 500 μ l of 10 mM dithiothreitol for gel-phase component assays. This dithiothreitol concentration did not interfere with the 7F10 or Alcian blue quantitative assays of the gel phase (data not shown).

Total protein. Total protein concentration in each sample was measured by a modified Lowry's method as previously described (26). Samples or human serum albumin (10 μ l) was placed in polystyrene microtiter plates (Dynatech Laboratories, Chantilly, VA) in duplicate. The optical densities (650 nm) were measured with a microtiter plate reader (Dynatech, Alexandria, VA). The protein concentrations in the samples were interpolated from regression analysis of the standard curve.

IgG. A new, direct, noncompetitive ELISA was based on previous methods (2, 26). Polystyrene microtiter plates were coated with 200 μ l of goat anti-human IgG F(ab')₂ fragment specific (Cappel, Cochranville, PA), diluted 1:1,000 in carbonate coating buffer (15 mM sodium carbonate, 35 mM sodium bicarbonate, and 0.02% sodium azide, pH 9.6), and incubated overnight at 4°C. The plates were washed with four volumes of pH 7.4 PBS-0.05% Tween 20 with a microplate washer. Dilutions of human IgG standard (Sigma, St. Louis, MO) and samples (200 μ l) were diluted 1:1,000 with diluent buffer (1% fetal calf serum and 0.02% sodium azide in PBS) and incubated for 2 h at room temperature. After the plates were washed, 200 μ l of goat anti-human IgG (γ -chain specific) alkaline phosphatase conjugate (Sigma) diluted 1:1,000 in PBS-Tween 20 were added to each well and incubated for 2 h at room temperature. After being washed, the plates were developed in the dark with 1 mg/ml of alkaline phosphatase substrate (Sigma) in 12 mM sodium carbonate-15.5 mM sodium bicarbonate-1 mM magnesium chloride, pH 8.6. The reaction was terminated by the addition of 3 N sodium hydroxide, and the optical densities (410 nm) were measured. Concentrations of IgG were interpolated from the standard curve.

Lysozyme. Lysozyme activity was measured by a turbidimetric assay based on the enzymatic hydrolysis of *Micrococcus lysodeikticus* (Sigma) cell walls as previously described (1, 2, 25, 26).

7F10-irm. 7F10 is a murine monoclonal antibody that was raised against human bronchial high-molecular-weight MGP synthesized by human bronchial explants (19). 7F10-irm is the MGP recognized by the 7F10 antibody. 7F10-irm is a marker of glandular and goblet cell exocytosis (1, 19). 7F10-irm was measured by ELISA as previously described (1, 19).

ABSM. Alcian blue 8GX is a copper-containing cationic isothiuronium dye (1,298.9 g/mol) that forms insoluble complexes with acidic carbohydrate polymers such as mucins and proteoglycans (24, 31). The nature of the ABSM in human nasal mucus has not been completely established but may consist of sulfated and sialylated mucins (20, 31). Samples for

the sol and gel phases were prepared as described in *Gel and sol phases*. Samples (25 μ l) or type I-S bovine submaxillary gland mucin standard (25 μ l, 0.12–250 μ g/ml; Sigma) was applied to positively charged nylon membranes (Boehringer Mannheim, Mannheim, Germany) in a 96-well dot-blot apparatus (Schleicher & Schuell, Keene, NH), and a low vacuum was applied (1, 24). The wells were washed two times with 250 μ l of 50 mM sodium bicarbonate. The membrane was washed two times for 5 min each in deionized water, blocked in 5% bovine serum albumin for 5 min, and then washed again in deionized water for 2 \times 5 min. The blot was stained with pH 2.5 3% Alcian blue 8GX (Poly Scientific, Bay Shore, NY) for 5 min, then washed three times in deionized water for 5 min each. The blot was dried in the dark at room temperature, covered with mineral oil (Sigma), and placed on a 96-well microtiter plate, and the optical densities (650 nm) were measured. Concentrations of ABSM were interpolated from the standard curve.

IL-8. Sol-phase samples were assessed by commercial IL-8 ELISA (CYTImmune Science, College Park, MD).

Statistical Methods

The data available for analysis consisted of the daily scores recorded for eight common symptoms of a cold (congestion, rhinorrhea, sneezing, sore throat, cough, malaise, headache, and chills); the measured concentrations of IL-8, total protein, IgG, and lysozyme in the daily lavage fluid; and the measured concentrations of 7F10-irm and ABSM in the gel and sol phases of the daily lavage fluid. After assays had been performed, data were assigned to infected and noninfected groups. The first statistical analysis of the infected group compared allergic and nonallergic subjects. Differences between the data sets from each day were evaluated with a two-tailed Mann-Whitney *U*-test. Allergy status had no effect on any of the variables tested. Therefore, data from all subjects were stratified into infected and noninfected groups. To demonstrate the serial changes brought on by RV-h infection, data from each day were compared with baseline (*day 0*) data for each individual with a two-tailed Wilcoxon single rank test (paired, nonparametric analysis). Differences between the infected and noninfected groups on each day were assessed by a two-tailed Mann-Whitney *U*-test. For all comparisons, statistical significance was established at $\alpha < 0.05$. Mean \pm SE is used throughout this presentation.

Data were plotted as the log of concentrations to accentuate changes.

RESULTS

Subjects

Allergic rhinitis was diagnosed in 32 subjects (21 men), whereas 48 subjects (22 men) had no evidence of allergic rhinitis (40% atopic). Virus infection was documented in 25 (78%) of the allergic and 36 (75%) of the nonallergic subjects (61 subjects infected out of 80 inoculated).

Symptoms

Infected subjects complained of significant nasal (sum of congestion, sneezing, and rhinorrhea), throat (sum of sore throat and cough), and general (sum of malaise, chills, and headache) symptoms beginning 48 h after RV-h inoculation (*day 2* scores). There was no difference in the severity or duration of these symptoms between allergic and nonallergic subjects (Fig. 1). Na-

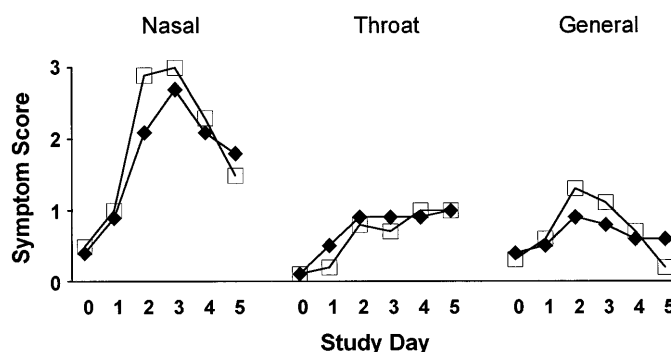


Fig. 1. Symptoms and atopy status. Average values of summary scores for nasal (sum of sneezing, congestion, and rhinorrhea; *left*), throat (sum of cough and sore throat; *middle*), and general (sum of headache, malaise, and chills; *right*) symptoms are shown on study *day 0* (preinoculation) and subsequent days for infected subjects with (\square) and without (\blacklozenge) nasal allergy.

sal and general symptom scores were maximal on *days 2* and *3*, then declined on *days 4* and *5*. Throat scores rose to a plateau on *day 2* and persisted throughout the period of cloister. Noninfected subjects had no changes in these scores (Fig. 2). Differences between infected and noninfected subjects were clearly demonstrated in all three categories on *day 2* and persisted for the nose and throat over the course of the study.

Mucus Constituents

As with symptoms, there were no significant differences between the allergic and nonallergic infected subjects for any constituent of the recovered lavage fluid (data not shown). Consequently, the data were combined for these groups, and the primary comparisons were made between infected and noninfected subjects. The mean concentrations of IL-8, total protein, IgG, and lysozyme in the lavage fluid and of ABSM and 7F10-irm in the sol and gel phases of the lavage fluid are shown for infected subjects in Table 1 and for noninfected subjects in Table 2.

IL-8

Low levels of IL-8 were detectable (>0.19 ng/ml) before rhinovirus inoculation in 39 of 61 subjects (64%), who subsequently demonstrated evidence of rhinovirus infection (Fig. 3). By *day 3*, 82% had detectable levels.

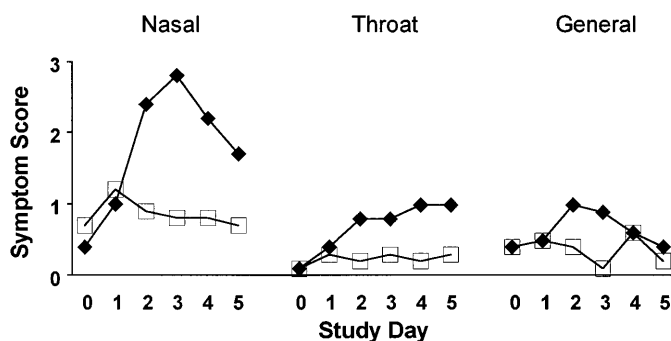


Fig. 2. Symptoms in infected (\blacklozenge) and noninfected (\square) subjects. Values are mean scores for nasal (*left*), throat (*middle*), and general (*right*) symptoms.

Table 1. *Infected subjects' nasal lavage fluid constituents*

Constituent	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
IL-8, ng/ml	1.72 ± 0.34	1.91 ± 0.34	3.37 ± 0.75§	11.85 ± 4.97§	9.48 ± 4.72*§	5.14 ± 2.04*§
Total protein, µg/ml	186 ± 19	255 ± 28	312 ± 31§	411 ± 43§	336 ± 37§	304 ± 33§
IgG, µg/ml	9.49 ± 1.71	11.19 ± 1.89	31.48 ± 7.86§	40.28 ± 8.77§	31.05 ± 7.89§	22.52 ± 4.30§
Lysozyme, µg/ml	20.6 ± 1.4	25.8 ± 1.7	28.1 ± 2.0	33.1 ± 3.0	28.6 ± 2.6	33.3 ± 2.6
7F10-irm, µU/ml						
Gel	154 ± 36	185 ± 37	209 ± 39	361 ± 61*§	320 ± 68†	304 ± 70†
Sol	720 ± 110	760 ± 120	930 ± 150†	1060 ± 180‡	810 ± 160	930 ± 160
ABSM, µg/ml						
Gel	154 ± 35	185 ± 37	209 ± 38†	360 ± 60‡	319 ± 68†	304 ± 70‡
Sol	103 ± 11	110 ± 10	126 ± 14	155 ± 14‡	152 ± 17	128 ± 14

Values are means ± SE. IL-8, interleukin-8; 7F10-irm, 7F10-immunoreactive mucin; ABSM, Alcian blue-staining mucoglycoprotein. *Significant difference compared with noninfected subjects (Table 2), $P < 0.05$ by Mann-Whitney U -test. Significant difference compared with day 0: † $P < 0.05$; ‡ $P < 0.01$; § $P < 0.01$ (all by Wilcoxon single rank test).

Their mean IL-8 concentration increased sevenfold on day 3. Concentrations were significantly different from baseline on days 2 ($P = 0.0004$), 3 ($P = 0.0001$), 4 ($P = 0.0002$), and 5 ($P = 0.0037$). IL-8 for infected subjects was significantly higher than that for the noninfected subjects on days 4 ($P = 0.041$) and 5 ($P = 0.0166$). Noninfected subjects had no significant change in IL-8 concentration.

Total Protein

In the infected subjects, total protein increased after inoculation and reached a maximal twofold increase on day 3 (Fig. 3). It then returned to baseline on days 4 and 5. The increases from baseline were significant on days 2 ($P = 0.0001$), 3 ($P < 0.0001$), 4 ($P < 0.0001$), and 5 ($P = 0.0003$). In the noninfected group, there was a slight trend for an increase in lavage fluid total protein concentration, but the difference was never significant compared with the baseline value. Because of the wide range of total protein concentration among individuals, there were no significant differences between the infected and noninfected groups on any day.

IgG

IgG increased fourfold on day 3 (Fig. 3). The ratio of IgG to total protein in the infected subjects increased from 5.1% before inoculation to 9.8% on day 3, then decreased to 7.4% on day 5. There were significant differences on days 2 ($P < 0.0001$), 3 ($P < 0.0001$), 4 ($P < 0.0001$), and 5 ($P < 0.0001$) versus baseline. This ratio must be interpreted with caution because the

proportions of each type of macromolecule in secretions changed each day. The Lowry assay may not have been sensitive to changes in this distribution because albumin was used as a standard and because mucin peptide backbones are less reactive than albumin by this colorimetric method.

In the uninfected subjects, IgG did not change on any day. The ratios of IgG to total protein in the noninfected subjects were 4.30 (baseline) and 4.25% (day 3).

Lysozyme

There were no significant changes in lysozyme in either set of subjects (Fig. 4). The ratio of lysozyme to total protein in the infected subjects decreased from 11.1% before inoculation to 8.0% on day 3 and did not change in the noninfected subjects (10.8% at baseline and 10.6% on day 3).

7F10-irm

The gel-phase 7F10-irm concentration rose twofold on days 3 through 5 for the infected subjects (Fig. 4). Concentrations did not change in the noninfected group. On day 3, the gel-phase 7F10-irm was significantly higher for the infected than for the noninfected group ($P = 0.015$).

Sol-phase concentrations of 7F10-irm tended to increase in both groups on days 2 and 3. However, significant differences from baseline were found only in the infected group.

The gel-to-sol ratio suggests partition of macromolecules between the sedimented material and the dis-

Table 2. *Noninfected subjects' nasal lavage fluid constituents*

Constituent	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
IL-8, ng/ml	0.73 ± 0.21	2.41 ± 1.08	1.15 ± 0.25	3.84 ± 2.49	1.57 ± 0.62*	1.31 ± 0.55*
Total protein, µg/ml	208 ± 20	243 ± 28	268 ± 38	292 ± 40	225 ± 33	262 ± 43
IgG, µg/ml	8.95 ± 2.37	9.78 ± 2.49	13.22 ± 3.30	12.42 ± 3.24	11.39 ± 3.60	13.97 ± 3.35
Lysozyme, µg/ml	22.39 ± 2.89	30.24 ± 3.58	30.62 ± 4.28	31.03 ± 3.79	27.01 ± 3.79	29.58 ± 3.84
7F10-irm, µU/ml						
Gel	108 ± 34	135 ± 40	153 ± 58	179 ± 63*	141 ± 57	204 ± 73
Sol	500 ± 140	300 ± 60	790 ± 300	830 ± 390	570 ± 250	570 ± 220
ABSM, µg/ml						
Gel	108 ± 33	134 ± 39	153 ± 56	179 ± 61	141 ± 57	204 ± 73
Sol	108 ± 28	112 ± 15	164 ± 29	136 ± 35	136 ± 26	118 ± 28

Values are means ± SE. *Significant difference compared with infected subjects (Table 1), $P < 0.05$ by Mann-Whitney U -test.

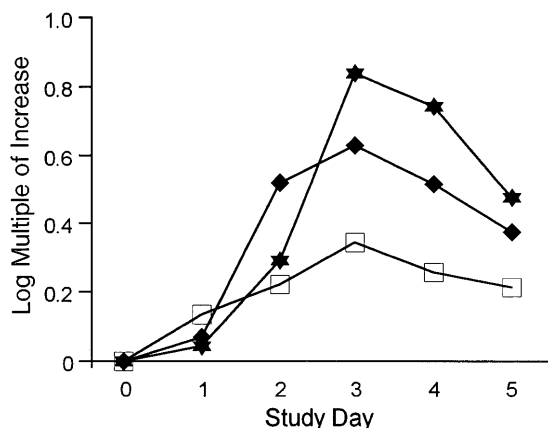


Fig. 3. Relative changes in mucus constituents of nasal lavage fluid. Logarithm of multiple of increase for each study day compared with day 0 is shown for total protein (□), IgG (◆), and interleukin-8 (★).

solved phase. This ratio was 0.27 ± 0.03 for 7F10-irm in noninfected subjects. Ratios were higher in infected subjects on days 3 (0.34), 4 (0.40), and 5 (0.33), a finding suggestive of mucus hypersecretion.

ABSM

The gel-phase ABSM concentration rose 1.7-fold on day 3 in the infected group but remained significantly elevated through day 5 (Fig. 4). There were no changes in the noninfected subjects.

Sol-phase ABSM showed a small, but significant, increase on day 3 in the infected subjects. The gel-to-sol ratio was 1.30 ± 0.11 for ABSM in noninfected subjects. Ratios were higher in infected subjects on days 3 (2.32), 4 (2.10), and 5 (2.38). Again, this is suggestive of mucus hypersecretion.

Summary of Changes

To gain an appreciation of the relative changes in marker concentrations in the infected group, IL-8 and IgG (Fig. 3) and glandular products (Fig. 4) were plotted as the logarithm of the average multiple of

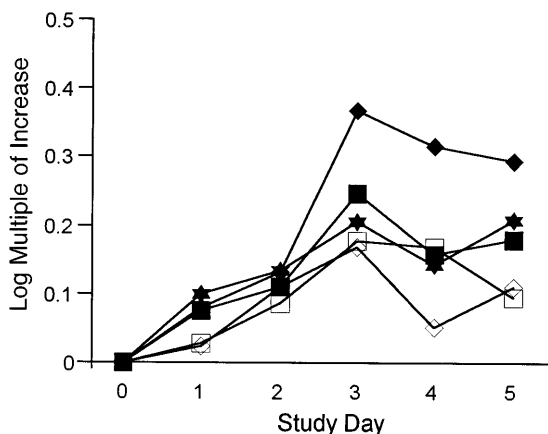


Fig. 4. Relative changes in glandular products in nasal lavage fluid. Logarithm of multiple of increase for each study day compared with day 0 is shown for lysozyme (★), 7F10-immunoreactive mucin in gel (◆) and sol (◇) phases, and Alcian blue-staining mucoglycoproteins in gel (■) and sol (□) phases.

increase for each marker as a function of time. IgG began to rise before the others (day 2), peaked on day 3, and then returned to baseline. IL-8 was maximal on days 3 and 4. Gel-phase 7F10-irm and ABSM were maximal on day 3 but were maintained as a plateau on days 4 and 5. Sol-phase 7F10-irm and ABSM were significantly increased on day 3 only. Lysozyme had no change.

DISCUSSION

The patterns and magnitudes of symptom expression for the subjects infected with RV-h were similar to those reported for infection with RV-39, influenza A, and Coxsackie virus infections (5–7, 17, 27). Congestion and rhinorrhea symptoms were maximal on days 3 and 4, with sore throats present from days 2 to 5.

In each of these experimental infection models, secretions increased progressively after inoculation, peaking on days 2 and 3 before subsiding toward prechallenge values by days 5–10. Vascular permeability increased rapidly and was maximal 3 days after inoculation. Increases in glandular products including sIgA (17), lysozyme (17), and the gel-phase 7F10-irm and ABSM (this study) tended to occur between days 3 and 5, suggesting a relatively delayed onset in glandular hypersecretion. The increase in sIgA (17) may reflect local antiviral IgA production with increased transport via serous cells. The increases in gel-phase 7F10-irm and ABSM suggest increased production and exocytosis of these mucins. It is unknown whether there is increased expression of the mucin backbone genes (29) or glandular or goblet cell hyperplasia.

Rhinorrhea has been perceived to be a beneficial host response to viral infection because large volumes of plasma and glandular macromolecules with antiviral and other protective functions are generated. However, the role of these macromolecules in promoting secondary bacterial colonization and subsequent mucosal infection is poorly understood. The benefits of mucus hypersecretion are clearly not appreciated by infected patients, who often seek symptomatic relief with α -adrenergic agonist, anticholinergic, and antihistaminic drugs. These symptomatic treatments do not appear to alter the pathogenesis of the viral illnesses.

Atopic status had no effect on these secretory processes because there were no differences in the concentration of mucus components from either vascular or glandular origins between the allergic and nonallergic groups in this and previous studies (7, 17). This finding suggests that the pathophysiology of the mucosal secretory responses after viral infections is similar in both groups. However, allergic subjects have significant differences in immune function after RV-39 infection (27), suggesting that immunologic responses to mucosal viral infections may differ in atopy.

A number of mediators have been found to be significantly increased in nasal secretions during rhinovirus infection. Bradykinin has been proposed as a mediator of the vascular permeability response (22). However, oral prednisone, which reduces bradykinin concentrations during the common cold, had no effect on signs or

symptoms (10). These data suggest that bradykinin may not be the primary mediator of rhinovirus-induced vascular permeability. Rather, the elevation of bradykinin in the common cold may be one consequence of the combination of increased plasma exudation plus increased activities of kininogenase enzymes. If so, then other mediators may be responsible for the prominent vascular leak. The absence of an effect of prednisone (11) suggests that inflammatory cascades that are not subject to regulation by glucocorticoids may be responsible for many rhinovirus symptoms.

Alternative candidate mediators include cytokines. IL-1 β , IL-6, IL-8, IL-11, tumor necrosis factor- α , regulated on activation normal T cell expressed and secreted, and macrophage inflammatory protein-1 α are significantly elevated during naturally acquired and experimental common colds (8, 9, 15, 16, 28). IL-8 is of particular interest because its levels increase rapidly and it parallels the time course of nasal symptoms and secretion of the neutrophil marker myeloperoxidase (16). IL-8 can induce acute obstruction to nasal airflow in atopic subjects (4), but this effect has not been assessed during rhinovirus infection. The temporal correlation of IL-8 production, rhinorrhea, nasal congestion, vascular permeability, and neutrophil infiltration suggests a cause-and-effect mechanism that should be addressed by future investigations (23, 28).

On the aggregate, the current and previous data from experimental RV-h and RV-39 infections and naturally acquired common colds suggest an early release of cytokines within 1–3 days, an increase in vascular permeability, and production of a plasma-rich exudate at the time of peak symptoms (2–4 days), followed by a subsequent rise in secretory IgA and gel-phase MGP production (after days 3–6). Although differences in immune responses have been described for allergic and nonatopic subjects, the current data indicate that there was no difference in mucus hypersecretory mechanisms for the two groups.

We thank Dr. Jack M. Gwaltney, (Department of Internal Medicine, University of Virginia, Charlottesville) for assistance with the methods and the rhinovirus *hanks* isolate and Dr. James H. Shelhamer and Carolea Logun (Clinical Center, National Institutes of Health, Bethesda, MD) for the 7F10 antibody.

This study was supported in part by grants from the National Institute of Mental Health (MH-50429) and the National Institute of Allergy and Infectious Diseases (AI-42403).

Address for reprint requests: J. N. Baraniuk, Division of Rheumatology, Immunology, and Allergy, Georgetown Univ. Medical Center, Gorman Bldg., GL-008, 3800 Reservoir Rd., N.W., Washington, DC 20007-2197.

Received 12 January 1998; accepted in final form 13 March 1998.

REFERENCES

1. Ali, M., J. Maniscalco, and J. N. Baraniuk. Spontaneous release of submucosal gland serous and mucous cell macromolecules from human nasal explants in vitro. *Am. J. Physiol. Cell Mol. Physiol.* 270 (Lung Cell. Mol. Physiol. 14): L595–L600, 1996.
2. Baraniuk, J. N., M. Shizari, M. Sabol, M. Ali, and C. B. Underhill. Hyaluronan is exocytosed from seromucous, but not mucous cells, of human nasal and tracheobronchial submucosal glands. *J. Invest. Med.* 44: 47–52, 1996.
3. Buchman, C. A., W. J. Doyle, D. Skoner, P. Fireman, and J. M. Gwaltney. Otolgic manifestations of experimental rhinovirus infection. *Laryngoscope* 104: 1295–1299, 1994.
4. Douglass, J. A., D. Dharmi, C. E. Gurr, M. Bulpitt, J. K. Shute, P. H. Howarth, I. J. Lindley, M. K. Church, and S. T. Holgate. Influence of interleukin-8 challenge in the nasal mucosa in atopic and nonatopic subjects. *Am. J. Respir. Crit. Care Med.* 150: 1108–1113, 1994.
5. Doyle, W. J., D. P. Skoner, P. Fireman, J. T. Seroky, I. Green, F. Ruben, D. R. Kardatzke, and J. M. Gwaltney. Rhinovirus 39 infection in allergic and nonallergic subjects. *J. Allergy Clin. Immunol.* 89: 968–978, 1992.
6. Doyle, W. J., D. P. Skoner, F. Hayden, C. A. Buchman, J. T. Seroky, and P. Fireman. Nasal and otologic effects of experimental influenza A virus infection. *Ann. Otol. Rhinol. Laryngol.* 103: 59–69, 1994.
7. Doyle, W. J., D. P. Skoner, M. V. White, F. Hayden, A. P. Kaplan, M. A. Kaliner, Y. Shibayama, and P. Fireman. Pattern of nasal secretions during experimental influenza virus infection. *Rhinology* 34: 2–8, 1996.
8. Einarsson, O., G. P. Geba, Z. Zhou, M. L. Landry, R. A. Panettieri, Jr., D. Tristram, R. Welliver, A. Metinko, and J. A. Elias. Interleukin-11 in respiratory inflammation. *Ann. NY Acad. Sci.* 762: 31–40, 1995.
9. Grunberg, K., M. C. Timmers, H. H. Smits, E. P. A. Deklerk, E. C. Dick, W. J. M. Spaan, P. S. Hiemstra, and P. J. Sterk. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavages in asthmatic subjects in vivo. *Clin. Exp. Allergy* 27: 36–45, 1997.
10. Gustafson, L. M., D. Proud, J. O. Hendley, F. G. Hayden, and J. M. Gwaltney, Jr. Oral prednisone therapy in experimental rhinovirus infections. *J. Allergy Clin. Immunol.* 97: 1009–1014, 1996.
11. Gwaltney, J. M., Jr., J. O. Hendley, G. Simon, and W. S. Jordan, Jr. Rhinovirus infections in an industrial population. I. The occurrence of illness. *N. Engl. J. Med.* 275: 1261–1268, 1966.
12. Gwaltney, J. M., and W. Jordan. Rhinovirus and respiratory illness in university students. *Am. Rev. Respir. Dis.* 93: 362–371, 1966.
13. Gwaltney, J. M., Jr., C. D. Phillips, R. D. Miller, and D. K. Riker. Computed tomographic study of the common cold. *N. Engl. J. Med.* 330: 25–30, 1994.
14. Hamparian, V. V. Rhinovirus. In: *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, edited by E. H. Lennette and N. J. Schmidt. Washington, DC: American Public Health Association, 1979, p. 535–575.
15. Hillerbrandt, M. H., K. L. Hopken, S. T. Roseler, and C. Bachert. Increased levels of proinflammatory cytokines and soluble intercellular adhesion molecule (ICAM) in nasal secretions of patients during natural acquired rhinovirus infection (Abstract). *Allergologie* 1: 43, 1996.
16. Hopken, K. L., M. H. Hillerbrandt, S. T. Roseler, and C. Bachert. Interleukin-8 and myeloperoxidase levels are elevated simultaneously in nasal secretions of patients during naturally acquired viral rhinitis (Abstract). *Allergologie* 1: 44, 1996.
17. Igarashi, Y., D. P. Skoner, W. J. Doyle, M. V. White, P. Fireman, and M. A. Kaliner. Analysis of nasal secretions during experimental rhinovirus upper respiratory infections. *J. Allergy Clin. Immunol.* 92: 722–731, 1993.
18. Johnston, S. L., G. Sanderson, P. K. Pattemore, S. Smith, P. G. Bardin, C. B. Bruce, P. R. Lambden, D. A. Tyrrell, and S. T. Holgate. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J. Clin. Microbiol.* 31: 111–117, 1993.
19. Logun, C., J. Mullol, D. Rieves, A. Hoffman, C. Johnson, R. Miller, J. Goff, M. Kaliner, and J. Shelhamer. Use of a monoclonal antibody enzyme-linked immunosorbent assay to measure human respiratory glycoprotein production in vitro. *Am. J. Respir. Cell Mol. Biol.* 5: 71–79, 1991.
20. Lundgren, J. D., and J. N. Baraniuk. Mucus secretion in inflammation. *Pulm. Pharmacol.* 5: 81–96, 1992.
21. McBride, T. P., W. J. Doyle, F. G. Hayden, and J. M. Gwaltney, Jr. Alterations of the eustachian tube, middle ear, and nose in rhinovirus infection. *Arch. Otolaryngol. Head Neck Surg.* 115: 1054–1059, 1989.
22. Naclerio, R. M., D. Proud, L. M. Lichtenstein, A. Kagey-Sobotka, J. O. Hendley, J. Sorrentino, and J. M. Gwaltney.

- Kinins are generated during experimental rhinovirus colds. *J. Infect. Dis.* 157: 133–142, 1988.
23. **Noah, T. L., F. W. Henderson, I. A. Wortman, R. B. Devlin, J. Handy, H. S. Koren, and S. Becker.** Nasal cytokine production in viral acute upper respiratory infection of childhood. *J. Infect. Dis.* 171: 584–592, 1995.
 24. **Pon, D. J., C. J. van Staden, and I. W. Rodger.** Hypertrophic and hyperplastic changes of mucus-secreting epithelial cells in rat airways: assessment using a novel, rapid, and simple technique. *Am. J. Respir. Cell Mol. Biol.* 10: 625–634, 1994.
 25. **Raphael, G. D., E. V. Jeney, J. N. Baraniuk, I. Kim, S. D. Meredith, and M. A. Kaliner.** Pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretions. *J. Clin. Invest.* 84: 1528–1535, 1989.
 26. **Raphael, G. D., S. D. Meredith, J. N. Baraniuk, H. M. Druce, S. M. Banks, and M. A. Kaliner.** The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am. Rev. Respir. Dis.* 139: 791–800, 1989.
 27. **Skoner, D. P., W. J. Doyle, E. P. Tanner, J. Kiss, and P. Fireman.** Effect of rhinovirus 39 (rv-39) infection on immune and inflammatory parameters in allergic and non-allergic subjects. *Clin. Exp. Allergy* 25: 561–567, 1995.
 28. **Teran, L.M., S. L. Johnston, J. M. Schroder, M. K. Church, and S. T. Holgate.** Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus induced asthma. *Am. J. Respir. Crit. Care Med.* 155: 1362–1366, 1997.
 29. **Verma, M., and E. A. Davidson.** Mucin genes: structure, expression and regulation. *Glycoconj. J.* 11: 172–179, 1994.
 30. **Wadowsky, R. M., S. M. Mietzner, D. P. Skoner, W. J. Doyle, and P. Fireman.** Effect of experimental influenza A virus infection on isolation of *Streptococcus pneumoniae* and other aerobic bacteria from the oropharynges of allergic and nonallergic adult subjects. *Infect. Immun.* 63: 1153–1157, 1995.
 31. **Whiteman, P.** The quantitative measurement of alcian blue-glycosaminoglycan complexes. *Biochem. J.* 131: 343–350, 1973.
 32. **Wilson, R., E. Alton, A. Rutman, P. Higgins, W. Al Nakib, D. M. Geddes, D. A. Tyrrell, and P. J. Cole.** Upper respiratory tract viral infection and mucociliary clearance. *Eur. J. Respir. Dis.* 70: 272–279, 1987.

