Rhinovirus-16 Colds in Healthy and in Asthmatic Subjects

Similar Changes in Upper and Lower Airways

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Rhinovirus (RV) infections appear to precipitate most asthma exacerbations. To investigate whether RV-16 induces different inflammatory changes in upper and lower airways of asthmatic and healthy subjects, we inoculated 10 nonatopic healthy and 11 atopic asthmatic adults with 2,000 TCID50 RV-16. Subjects recorded symptoms and peak flow daily; and they underwent spirometry, methacholine challenge (PC_{20}), nasal lavage, and sputum induction at baseline and on Days 2, 4, 15, and 29 d after inoculation. One asthmatic subject developed an exacerbation requiring prednisone treatment 5 d after inoculation. The cold symptom severity (Jackson score) did not differ between groups. During the cold, asthma symptoms increased slightly from baseline in the asthmatic group; and PC_{20} decreased in the healthy group. However, peak flow, bronchodilator use, and spirometry did not change in either group. At baseline, asthmatics had higher neutrophils, eosinophils, and interleukin (IL)-6 in nasal lavage. After inoculation, both groups developed significant increases in nasal neutrophils, IL-6 and IL-8, and modest increases in sputum neutrophils and IL-6, but not IL-8. However, these changes did not differ between groups. IL-5, interferon-γ, and RANTES were detected only in nasal lavages from two asthmatic subjects, who had the most severe colds. IL-11 was not detected in any sample. We conclude that inflammatory responses of upper and lower airways during RV-16 colds are similar in asthmatic and healthy subjects, and that RV-16 infection is not by itself sufficient to provoke clinical worsening of asthma. Fleming HE, Little FF, Schnurr D, Avila PC, Wong H, Liu J, Yagi S, Boushey HA. Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. AM J RESPIR CRIT CARE MED 1999;160:100–108.

Viruses responsible for the common cold, particularly rhinoviruses, are frequently isolated from upper airway secretions during asthma exacerbations (1, 2). Investigations of possible mechanisms of this association have involved study of pulmonary function and airway inflammation in one of three circumstances: asthmatic patients suffering exacerbations (1–3), patients with symptomatic natural common colds (4–7); and subjects inoculated with a strain of rhinovirus (8–21). In a study of the first of these types of investigation, we found high numbers of neutrophils and eosinophils and high concentrations of interleukin (IL)-6, IL-8, neutrophil elastase, albumin, and a mucinlike glycoprotein in the sputum spontaneously expectorated by patients presenting with severe asthma attacks, particularly in those who reported cold symptoms as preceding their attack (3). We inferred that the mechanisms of asthma exacerbations associated with upper respiratory infections (URIs) may involve recruitment of neutrophils (as well as eosinophils) into the airways.

One study of asthmatics suffering community-acquired URIs without asthma exacerbations found modest increases in neutrophils in bronchial lavage fluid, and in eosinophils and T-lymphocytes in biopsies of the bronchial mucosa (6). Other studies have shown increased levels of IL-1 beta, tumor necrosis factor alpha (5), IL-6 (22), IL-8 (7), and IL-11 in nasal secretions obtained during community-acquired URIs (23). Some cytokines in nasal secretions were found to correlate with the severity of symptoms such as IL-8, a potent neutrophil chemoattractant, which correlated with the severity of cold symptoms (7); and IL-11, which was found in highest concentrations in nasal secretions from children who had wheezing during the colds (23).

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The findings of these "field studies" have been extended by studies of the effects of experimental inoculation of subjects with different respiratory viruses (8, 10, 16, 17). These have shown that experimental colds consistently cause typical cold symptoms and an increase in neutrophil numbers in nasal lavage (10, 12, 17, 21, 24), and inconsistently cause bronchoconstriction and an increase in bronchial reactivity (8, 10, 15–17, 19, 24). Using sputum induced by hypertonic saline to assess lower airway inflammation, Grunberg and colleagues (20, 21) showed that nasal inoculation with rhinovirus serotype 16 (RV-16) caused increases in concentrations of IL-6, IL-8, and eosinophil cationic protein in induced sputum and in IL-8 concentration in nasal lavage fluids. They did not find an increase in the proportion of neutrophils in induced sputum, but they did find an increase in the proportion of neutrophils containing IL-8.

Taken together, these studies have shaped the hypothesis that viral infection of the respiratory epithelium stimulates the production of proinflammatory cytokines, which attract and activate inflammatory cells in the airway mucosa, where their products cause edema, neural stimulation, and mucus secretion (20, 21, 25). Rhinoviruses, once thought only to infect the upper airway, are now also thought possibly to infect the lower airways, where the effects of inflammatory cell mediators include contraction of airway smooth muscle and an increase in bronchial reactivity (18, 19).

TABLE 1

BASELINE CLINICAL CHARACTERISTICS OF THE STUDY SUBJECTS

Subject No.	Sex	Age (*yr*)	FEV_1 (*L*)	FEV_1 (*% pred*)	FEV_1/FVC Ratio	$PC_{20}FEV_1$ (*mg/ml*)	RV Ab Titer*
Healthy							
1	F	33	3.1	107	83.8	83	0
2	F	32	3.9	126	78	29	0
3	M	25	3.7	86	80.4	93	0
4	F	19	4.4	126	84.6	90	1
5	F	30	4.4	126	88	23	0
6	F	34	3.1	107	91.2	124	0
7	M	19	5.6	124	90.3	112	0
8	F	43	3.5	106	83.3	91	1
9	F	31	2.9	104	76.3	112	0
10	F	27	3.6	106	83.7	22	0
Mean		29.3	3.82	112	83.9	78	
Asthmatic							
11	M	36	3.2	89	72.7	0.41	1
12	F	34	2.9	97	82.9	0.75	0
13	M	31	4.0	83	67.8	0.39	1
14	M	30	3.8	97	77.6	1.44	0
15	F	42	3.2	107	69.6	0.71	0
16	M	27	3.9	81	62.7	0.30	0
17	F	46	2.5	100	69.4	0.13	0
18	F	24	3.4	100	72.3	0.49	1
19	F	43	2.0	87	76.9	1.57	1
20	M	34	3.4	77	73.9	0.99	1
21†	F	56	1.6	80	64	0.54	0
Mean		34.7	3.23	92	72.6	0.72	
p Value‡	NS	NS	0.08	0.003	0.0007	0.0001	

Definition of abbrevitions: $PC_{20}FEV_1$ = concentration of methacholine causing a 20% fall in FEV_1 (the maximum dose of methacholine was 80 mg/ml. $PC_{20}FEV_1 > 80$ mg/ml were extrapolated based on slope and cumulative breath units); NS = nonsignificant ($p < 0.05$).

* RV Ab Titer is the reciprocal of the highest dilution of serum causing neutralization of 100 TCID50 of RV-16. Titer = 0, means failure of undiluted serum to neutralize RV-16.

† Asthmatic subject who developed an exacerbation needing oral prednisone. She is excluded from the data analysis (mean and p value calculations).

‡ Comparison between healthy and asthmatic subjects with Fisher's exact test (for sex) and Mann-Whitney rank sum test.

This hypothesis is consistent and plausible, but it does not provide an explanation for the differences in the clinical and physiologic consequences of rhinovirus infection in healthy persons, who develop only cold symptoms, and asthmatics, who may develop lower airway symptoms as well. In this study, we investigated the hypothesis that the upper (or nasal) airway responses would be similar, but the lower (or bronchial) airway responses would be different in healthy and asthmatic subjects inoculated with RV-16. The specific end-points examined were the severity of symptoms referable to the upper and lower airways, tests of maximal airflow and bronchial reactivity, and the cellular and biochemical content of nasal lavage and induced sputum in 10 asthmatic and 10 healthy adults. The biochemical content studied in the nasal lavage and induced sputum samples were selected to assess production of cytokines by airway epithelial (IL-6, IL-8) and stromal (IL-11) cells, and the production of eosinophil chemoattractants and activators (IL-5, RANTES, and GM-CSF). We also measured IL-5 as a marker of T helper 2 (Th2)-like activity and interferon gamma as a marker of T helper 1 (Th1)-like activity.

METHODS

Subjects

Ten healthy and 11 asthmatic adults were recruited by advertisement (Table 1). Inclusion criteria were age between 18 and 55 yr and no previous exposure to RV-16 defined as a serum titer of neutralizing antibody to RV-16 less less than 2. Exclusion criteria were smoking in the previous year or more than 5 pack-years lifetime, cold symptoms, or use of any corticosteroid in the previous 6 wk or any illness other than asthma requiring therapy through the course of the study. The healthy subjects had no history of allergic rhinitis, ≤ 1 positive reactions to allergen skin prick testing, and had normal spirometry and normal bronchial reactivity to methacholine ($PC_{20} > 8$ mg/ml). Asthmatic subjects were required to be only on "as needed" beta-agonist inhalation treatment, to be atopic with ≥ 2 positive skin test reactions to allergen extracts, to have bronchial hyperreactivity to methacholine ($PC_{20} < 8$ mg/ml), to have a prebronchodilator $FEV_1 > 70\%$ predicted at baseline, and to have not used any nasal, inhaled, or oral corticosteroid within the previous 3 mo. All subjects signed informed consent forms approved by the Committee on Human Research of the University of California San Francisco (UCSF).

Protocol

Subjects kept diaries of cold and asthma symptoms and of peak expiratory flow (*see below*). During study visits, at baseline and on Days 2, 4, 8, 15, and 29 after RV-16 inoculation, subjects underwent spirometry, methacholine challenge, nasal lavage, and sputum induction, as shown in Figure 1. Day 0 is the day of the first RV-16 inoculation.

Allergen Skin Prick Test

The test was performed as previously described (26) using 12 allergen extracts (Greer Laboratories, Lenoir, NC): *Dermatophagoides pteronyssinus, D. farinae*, cat, dog, cockroach, Western oak mix, Bermuda-Johnson grass mix, short ragweed, plantain sorrel mix, alternaria, *Cladosporium herbarum*, aspergillus mix, and negative control. Positive control was histamine base 1.8 mg/ml (Allermed Laboratories, San Diego, CA). Skin reactivity was assessed 15 min after the prick and considered positive if a ≥ 3 mm wheal surrounded by erythema developed and negative if no wheal developed.

Bronchial Reactivity to Methacholine

Spirometry was performed according to ATS criteria with a dry-rolling seal spirometer (Ohio 840; Ohio Medical Products, Atlanta, GA) (27). Values for FEV_1 and FVC were selected from the best of three maneuvers. Bronchial reactivity to methacholine was assessed as de-

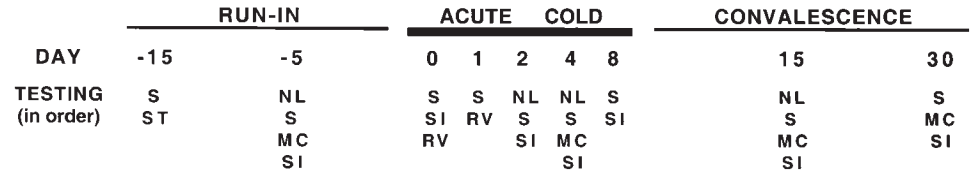

Figure 1. Diagrammatic representation of the 6-wk parallel group trial to compare upper and lower airway responses to nasal inoculation with RV-16 in asthmatic and healthy subjects. During run-in period, subjects were characterized by spirometry (S), skin test (ST), nasal lavage (NL), methacholine reactivity (MC), and sputum induction (SI). In the third week, subjects were inoculated with rhinovirus (RV) 16 on two consecutive days (Days 0 and 1) and were followed for 4 wk with repeated testing. Tests were performed in the order they are shown in the diagram.

scribed previously (26). Briefly, five breaths of each concentration of methacholine chloride in Ca- and Mg-free phosphate-buffered saline solution (0.078 to 80 mg/ml) were progressively administered in doubling concentrations and at 5-min intervals using a DeVilbiss #646 nebulizer (DeBillbiss, Somerset, PA) connected to a breath-activated French-Rosenthal Dosimeter (Baltimore, MD). Spirometry was performed 3 min after each concentration was delivered until a 20% or greater fall in FEV_1 was achieved. The provocative concentration causing a 20% fall in FEV_1 (PC_{20}) was calculated by log-linear interpolation, or if a 20% fall in FEV_1 was not achieved, by extrapolation using slope and cumulative methacholine dose (28).

Symptom Score and Peak Flow Monitoring

Subjects recorded each evening their common cold and asthma symptoms; and twice a day they recorded peak expiratory flow (PEF) and the number of puffs of albuterol MDI taken.

Cold symptoms were assessed as proposed by Jackson and colleagues (29) grading from 0 to 3 (absent, mild, moderate, or severe) eight symptoms: nasal discharge, nasal congestion, sneezing, cough, malaise, throat discomfort, fever/chills, and headache. The Jackson cold score was calculated by summing all scores from Day 1 to Day 6 after the inoculation, minus the sum of scores over the 6 d before the first inoculation (Days -1 to -6). The clinical criteria for diagnosing a cold were *either* a Jackson cold score > 13 combined with either increased rhinorrhea for ≥ 3 d after RV inoculation or the subject's perception of a cold; *or* if the cold score was ≤ 13, the combination of the perception of a cold and the increase in rhinorrhea (29). A Jackson score ≥ 20 defined a severe cold, between 14 and 19 a moderate cold, and ≤ 13 a mild cold.

The validated asthma symptom score (30) graded on an ordinal scale from 0 (none) to 10 (extremely severe) five symptoms: shortness of breath, chest tightness, wheezing, cough, and sputum/phlegm.

Subjects were instructed to measure peak flow in triplicate with a Mini-Wright peak flow meter within 15 min after waking each morning and before retiring each evening, but > 4 h after their last dose of inhaled albuterol, and to record the values in their diaries.

Rhinovirus 16 Procedures

The strain of rhinovirus-16 was generously provided by William Busse, M.D., and Elliot Dick, Ph.D. (University of Wisconsin, Madison, WI) as cell culture passage 2, which we expanded on embryonic lung fibroblasts WI-38 without bovine serum using standard methods of culture. We confirmed that the virus grown was rhinovirus 16 in the Virology Laboratory, Moffitt Hospital, UCSF; and we followed the recommended safety procedures (31, 32) to ensure the absence of other pathogens. Our methods were reviewed and approved by the Biosafety Committee of the UCSF. Titration of RV-16 in the inocula and in nasal lavage fluids was expressed as tissue culture infective dose 50% per ml (TCID50/ml). Inoculation with RV-16 was performed by instilling with a transfer pipette 0.5 ml of a 1,000 TCID50/ml suspension into each nostril on two consecutive days (Figure 1). To quantify the titer of rhinovirus-neutralizing antibodies in serum, duplicate serial doubling dilutions of serum in medium were mixed with equal volumes of 100 TCID50 of RV-16 challenge. The control samples included serum without RV-16 for serum toxicity control, and tenfold serial dilutions of the RV-16 challenge alone to confirm viability and the TCID50 titer of the RV-16 challenge. The mixtures were allowed to react for 30 min at room temperature. Then, 100 μl of each sample were added to wells containing a monolayer of human fetal diploid lung cell (VDRL, Berkeley, CA). The appearance of cytopathic effect was observed for 7 d or until virus titer reached 100 TCID50 in the control wells. Titration of RV-16 in nasal lavage fluid was performed likewise using serial 10-fold dilutions. Titer is expressed as the reciprocal of the highest dilution to cause neutralization. Infection by RV-16 was documented by either recovery of RV-16 from nasal lavages or seroconversion of neutralizing antibodies to RV-16 (Titer ≥ 4).

Nasal Lavage and Sputum Induction.

Nasal lavage was performed by instilling 5 ml of warmed (37° C) normal saline into each nostril, one at a time, with the subject's head elevated 45 degrees. Subject held the saline solution for 20 s by silently holding the sound of the letter K, which closes the posterior nasal passage. Then, the subject leaned forward and expelled the nasal contents into a plastic cup (10, 11, 33). We performed sputum induction as previously reported (26). Briefly, the subjects inhaled 4 puffs (360 μg) of albuterol, and 10 min later performed spirometry and peak flow. Next, while wearing a noseclip, the subjects breathed through a mouthpiece nebulized 3.0% saline solution (150 ml in the reservoir) for 20 min (Ultra-Neb 99 nebulizer, output of 6.1 ml/min, particle mean diameter of 2.4 μm; DeVilbiss). At 4-min intervals subjects cleared their mouth by spitting saliva into a plastic cup (discarded saliva), coughed sputum into another plastic cup (induced sputum sample), and then performed peak flow. Sputum induction was terminated earlier if peak flow and FEV_1 fell below 80% of the postalbuterol values.

Nasal lavage and induced sputum samples were processed as previously described (26). Briefly, an equal volume of 6.5×10^{-4} M dithiothreitol (Sputolysin concentrate, cat. no. 09368; Behring Diagnostics Inc.,Westwood, MA) diluted 1:10 in NaCl 0.9% was added to the sample, mixed on a vortex, and placed in a shaking water bath at 37° C for 15 min, during which the sample was further homogenized every 5 min with a large-bore transfer pipette. Then, 10 μl were used for total cell count in a hemocytometer, and 25,000 to 40,000 cells per slide were used to make four cytosmears per sample. Slides were stained with Leukostat (Fisher Diagnostics, Pittsburgh, PA). The rest of the sample was centrifuged at 2,000 rpm for 5 min and the supernatant was aliquoted and frozen at -70° C for future cytokine measurements. Differential count was obtained from 500 nonsquamous cells or, for some nasal lavage samples, after counting all four slides per sample.

Quantification of Cytokines

IL-5, IL-6, IL-8, IL-11, RANTES, GM-CSF, and interferon-γ were measured by EIA according to manufacturer's instructions (Quantikine kits from R&D Systems, Minneapolis, MN). The sensitivity of those tests were, respectively: 3.0, 0.7, 10.0, 8.0, 5.0, 0.36, and 3.0 pg/ml. Recovery of IL-11 added to sputum samples before processing was 80%.

Data Analysis

Data were analyzed with nonparametric tests using StatView (BrainPower Inc., Calabasas, CA) and Stata (Stata Corporation, College Station, TX) software. Friedman's test was used to analyze repeated measures, and when significant, within-group comparisons were ana-

lyzed with Wilcoxon's signed rank test, and between-group comparisons with the Mann-Whitney rank sum test. Correlations were analyzed with Spearman's rank correlation test. Proportions were compared with Fisher's exact test. A p value < 0.05, using two-tailed tests, was considered statistically significant. Values in tables are expressed as mean and standard deviation for FEV_1 and PC_{20}, and median and range for sputum and nasal lavage variables.

RESULTS

The ten healthy subjects enrolled completed the study. A single asthmatic subject developed clinical worsening of asthma 5 d after RV inoculation; she was treated with oral corticosteroid and withdrawn from the study. Data from this subject are not included in data analysis, but they are reported separately. To compensate for her withdrawal, an eleventh asthmatic subject was enrolled. The results presented for the two groups thus reflect data from 10 healthy and 10 asthmatic subjects who completed the protocol (Table 1).

RV-16 Infection and Cold Symptoms

Nasal inoculation with RV-16 led to infection in all but one healthy subject, as diagnosed by recovery of RV-16 from nasal lavage fluid on Days 2 or 4 after inoculation (18 of 20) and/or a $\geq$ 4-fold rise in serum neutralizing antibodies titer obtained 4 wk after inoculation (13 of 20) (Table 2). The healthy subject without microbiologic confirmation of infection (Subject 10) perceived a severe common cold based on Jackson's criteria. According to Jackson's criteria, which was validated in more than 1,000 subjects infected with rhinovirus, a cold score > 13 with perception of a cold confirms a symptomatic experimental cold and correlates with microbiologically documented rhinovirus infection (29). One asthmatic subject (Subject 20), although infected by RV-16, did not perceive a cold and had a negative cold score probably because of increased nasal symptom scores caused by allergic rhinitis during the baseline week. The intensity of cold symptoms did not differ between the two groups.

Asthma Symptoms and Pulmonary Function

Among the healthy subjects, the RV-16-induced upper respiratory infections were associated with no significant changes in asthma symptoms and no decrease of $> 15\%$ in AM PEF during the week after inoculation. The healthy subjects showed no significant change from baseline in FEV_1 (Figure 2, *top panel*), but they developed a significant increase in methacholine reactivity on Day 4 after inoculation compared with baseline (doubling dose $= -0.66 \pm 0.2$ [mean $\pm$ SEM], $p = 0.01$, and $p = 0.03$ versus asthma group) (Figure 2, *bottom panel*).

TABLE 2

COLD SYMPTOM SCORE, DURATION OF RHINORRHEA, MAXIMAL NASAL LAVAGE TITER OF RV-16, AND SEROCONVERSION IN HEALTHY AND ASTHMATIC SUBJECTS INOCULATED WITH RV-16

Subject No.	Cold Symptom Score	Duration of Rhinorrhea (*d*)	Perceived a Cold	RV-16 Titer in Nasal Lavage* (10^x/*ml*)	Postinfection Ab Titer to RV-16†
Healthy					
1	46	5	Yes	4.5	≥ 8
2	28	6	Yes	4.5	≥ 8
3	13	0	Yes	3.5	4
4	31	4	Yes	3	2
5	36	6	Yes	5	4
6	12	2	Yes	1	≥ 8
7	15	3	Yes	3	1
8	25	6	Yes	5.5	2
9	24	5	Yes	3.5	2
10	30	2	Yes	NG	2
Mean	26.2	3.9	10/10	3.35	5/10‡
Asthmatic					
11	31	5	Yes	3	≥ 8
12	31	6	Yes	3.5	≥ 8
13	24	6	Yes	4	1
14	31	5	Yes	3.7	≥ 8
15	26	4	Yes	1.5	≥ 8
16	54	6	Yes	5.5	4
17	39	6	Yes	5.5	1
18	34	5	Yes	NG	≥ 8
19	30	3	Yes	4.5	≥ 8
20	−10	0	No	2.5	≥ 8
21§	28	> 3	Yes	3.5	≥ 8
Mean	29.0	4.6	9/10	3.72	8/10‡
p Value¶	NS	NS	NS	NS	NS

Definition of abbreviations: NG = no growth; NS = nonsignificant ($p > 0.05$).

* Titer in TCID50 from 0.1 ml of nasal lavage fluid (*see* METHODS).

† Titer is the reciprocal of the highest dilution of serum causing neutralization of 100 TCID50 of RV-16.

‡ Rate of seroconversion (fourfold or higher increase in titer).

§ Asthmatic subject who was withdrawn because of an exacerbation and whose data are excluded from analysis (mean and p value calculations).

¶ Comparison between healthy and asthmatic subjects with Fisher's exact test and Mann-Whitney rank sum test.

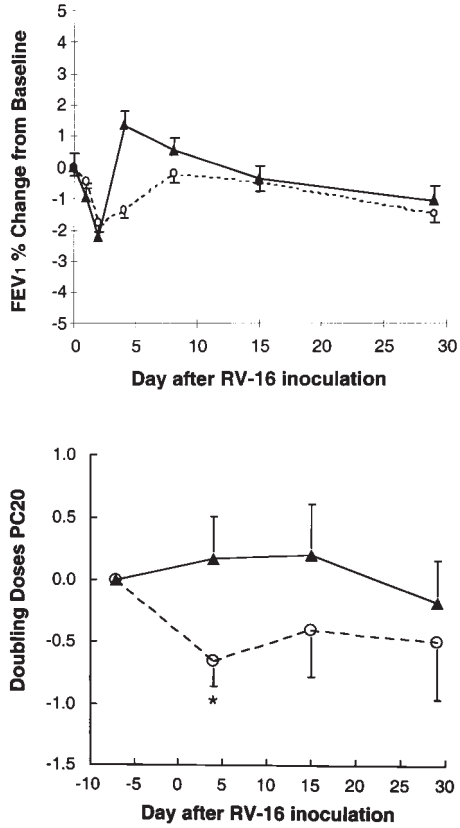

Figure 2. Changes in pulmonary function during RV-16 cold in healthy (*open circles*) and asthmatic (*closed triangles*) subjects. *Top panel* shows changes in FEV_1 as percentage from baseline (mean $\pm$ SEM). *Bottom panel* shows changes in provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) as doubling doses from baseline (mean $\pm$ SEM). There was no significant change in FEV_1 after RV-16 inoculation on Days 0 and 1. *PC_{20} reduced significantly on Day 4 in the healthy subjects compared with baseline ($p = 0.01$) and with asthmatic subjects (0.03).

TABLE 3

PULMONARY FUNCTION, NASAL LAVAGE, AND INDUCED SPUTUM VARIABLES IN HEALTHY AND ASTHMATIC SUBJECTS INOCULATED WITH RV-16*

							p Value§		
							Healthy × Asthmatic		Paired
Variable	Group	Baseline†	Acute Cold‡	Day 8	Day 15	Day 29	Baseline	Acute Cold	Baseline × Acute Cold
Pulmonary function									
FEV_1, L	Healthy	3.78 ± 0.8	3.74 ± 0.8	3.78 ± 0.8	3.76 ± 0.8	3.73 ± 0.8	NS	NS	0.07
	Asthmatic	3.13 ± 0.6	3.12 ± 0.7	3.16 ± 0.7	3.13 ± 0.7	3.13 ± 0.8			NS
PC_{20}, mg/ml	Healthy	78 ± 39	52 ± 32	–	75 ± 63	76 ± 63	0.0002	0.0002	0.01
	Asthmatic	0.72 ± 0.5	0.87 ± 0.7	–	1.07 ± 1.1	0.81 ± 0.8			NS
Nasal lavage									
Neutrophils × 10^6	Healthy	0.1 (0–0.2)	7.9 (0–156)	–	0.8 (0–6)	–	0.01	NS	0.009
	Asthmatic	2.5 (0.002–6.1)	4.4 (0.007–53.6)	–	2.2 (0.003–30)				0.09
Eosinophils × 10^6	Healthy	0 (0–0.04)	0.006 (0–2)	–	0 (0–0.001)	–	0.004	0.006	0.07
	Asthmatic	0.04 (0–2.6)	0.48 (0–3.4)	–	0.11 (0–8.3)	–			NS
IL-6, pg/ml	Healthy	4.5 (0–8)	310 (0–5,120)	–	14 (0–112)	–	0.009	NS	0.005
	Asthmatic	16.5 (0–150)	193 (0–4,800)	–	23.5 (0–190)	–			0.012
IL-8, ng/ml	Healthy	0.8 (0–4.8)	2.4 (0–9.1)	–	1.9 (0–5.7)	–	0.06	NS	0.005
	Asthmatic	1.7 (0.2–19.6)	2.2 (0.1–22.4)	–	1.3 (0.2–20.8)	–			NS
Induced sputum									
Neutrophils, %	Healthy	43.3 (19–76)	56 (14–75)	48 (26–89)	36 (14–79)	43 (29–63)	NS	NS	NS
	Asthmatic	34 (13–95)	48 (22–84)	41 (13–85)	42 (9–72)	42 (14–87)			0.03
Eosinophils, %	Healthy	0 (0–1.3)	0 (0–0.9)	0 (0–0.6)	0 (0–0.4)	0.2 (0–0.4)	0.0002	0.0002	NS
	Asthmatic	3.2 (0–34)	2.0 (0–12)	2.5 (0–11)	2.1 (0–14)	2.6 (0–9)			0.09
IL-6, pg/ml	Healthy	180 (0–480)	267 (16–656)	228 (10–664)	185 (18–856)	185 (13–380)	0.07	NS	0.005
	Asthmatic	103 (9–272)	191 (11–2,080)	130 (27–332)	109 (13–310)	108 (15–228)			0.005
IL-8, ng/ml	Healthy	2.9 (0.4–5)	3.8 (0.7–5.8)	4.3 (0.7–5.7)	3.3 (0.8–5.2)	2.7 (0.8–4)	NS	NS	0.03
	Asthmatic	3.1 (0.6–26.4)	3.4 (0.5–12.4)	3.1 (0.9–5.2)	2.9 (0.5–4)	2.6 (520–4.6)			NS

* Values are mean ± SD for pulmonary function variables and median (range) for the other variables.
† Baseline values are the mean of measurements taked during run-in period.
‡ Acute cold values are the mean of Days 2 and 4 after the inoculation (Days 0 and 1).
§ p Value by Mann-Whitney rank sum test when comparing healthy versus asthmatic subjects at baseline and during the acute cold, and by Wilcoxon's sign rank test when comparing baseline versus acute cold within the same group.

Among the 10 asthmatic subjects who completed the protocol, RV-induced URIs were associated with minimal evidence of change in lower airway function. Asthma symptoms increased slightly, but significantly, from a daily average of 4.9 (range, 0 to 12.7) in the baseline week to 8.7 (range, 1.4 to 22.1; $p < 0.025$) in the week of acute common cold, but neither mean daily "as needed" use of albuterol nor PEF changed significantly. During the cold, FEV_1 did not change from baseline (Figure 2, *top panel*), but in contrast to the healthy group, PC_{20} methacholine did not change from baseline (doubling dose = +0.17 ± 0.3, $p = 0.5$) (Figure 2, *bottom panel*).

Nasal Lavage

Analysis of nasal lavage fluid revealed the expected changes in inflammatory cell content (Table 3 and Figures 3 and 4). The total number of neutrophils increased sharply and similarly in both groups on the second and fourth day after inoculation (overall mean from 1.5×10^6 at baseline to 20×10^6 on Day 4, $p = 0.024$), returning toward baseline 2 wk later (Figure 3, *top panel*). The total number of eosinophils was greater in the nasal lavage fluid from the asthmatic subjects on all days (Figure 3, *bottom panel*), and no significant changes occurred after RV inoculation in either group.

The rise in total neutrophil number was associated with increases in the concentrations of IL-6 in nasal lavage fluid from both groups of subjects (Figure 4, *top panel*). The IL-6 levels peaked on the second day after inoculation and returned toward baseline 2 wk later (overall means for both groups were 19.7 pg/ml at baseline and 932.5 pg/ml on Day 2, $p = 0.0003$). At baseline, interleukin-8 levels in nasal lavage from the asthmatic subjects were insignificantly higher than in the healthy subjects, and increased significantly only in the healthy subjects (Figure 4, *bottom panel*), peaking on Day 4. The other cytokines measured were detected infrequently. Interleukin-5, interferon-γ, and RANTES were detected only in the nasal lavage fluid from two asthmatic subjects (Subjects 16 and 17 in Table 2) during the acute phase of rhinovirus cold. These subjects had the highest acute cold symptom scores, the greatest increases in nasal lavage neutrophils and eosinophil numbers, and the highest levels of IL-8 of the asthmatic subjects. Interleukin-11 and GM-CSF were not detected in nasal lavage from any subject.

Some observed changes in the outcome variables correlated with each other and with symptoms. Analyzing all subjects together with mean values of Days 2 and 4 after inoculation (acute cold), cold symptoms correlated with nasal neutrophil numbers ($r = 0.45$, $p = 0.045$ by Spearman's correlation) and with nasal IL-8 levels ($r = 0.52$, $p < 0.02$), but not with IL-6 ($r = 0.22$, $p = 0.36$). Similarly, total numbers of neutrophils correlated with nasal IL-8 levels ($r = 0.78$, $p < 0.0001$) and with nasal IL-6 levels ($r = 0.63$, $p = 0.003$).

Induced Sputum

Induced sputum samples showed small changes in markers of inflammation (Table 3 and Figures 5 and 6). The percentage of neutrophils, expressed as a percentage of nonsquamous cells, increased modestly and similarly in both groups ($p = 0.006$ by Friedman's test for both groups analyzed together).

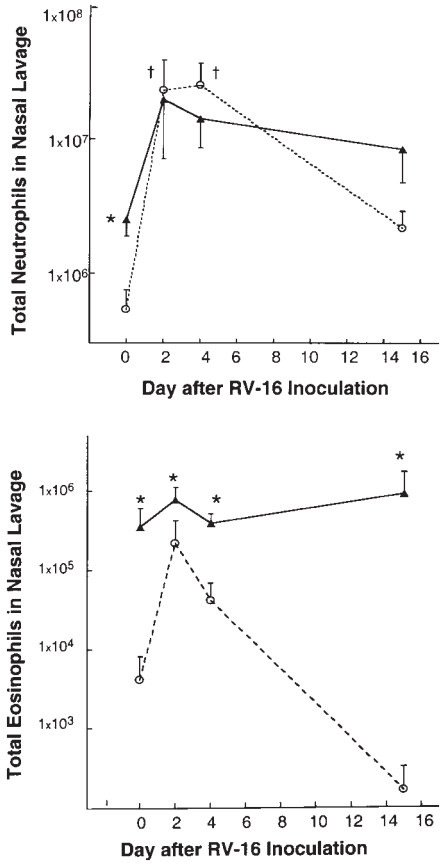

Figure 3. Neutrophils and eosinophils in nasal lavage from healthy (*open circles*) and asthmatic (*closed triangles*) subjects inoculated with RV-16 on Days 0 and 1. *Top panel* represents neutrophils and *bottom panel* represents eosinophils, both expressed as mean ± SEM of absolute number per nasal lavage. In healthy subjects, the neutrophil number was significantly lower than in asthmatic subjects at baseline (*top panel*, *$p = 0.01$), and increased during acute cold compared with baseline (*top panel*, †$p \leq 0.02$). Taken together, the two groups had a significant increase in neutrophils during the acute cold (Days 2 and 4, *see text*). Throughout the study, asthmatic subjects had higher number of eosinophils in nasal lavage than did healthy subjects (*bottom panel*, *$p \leq 0.01$).

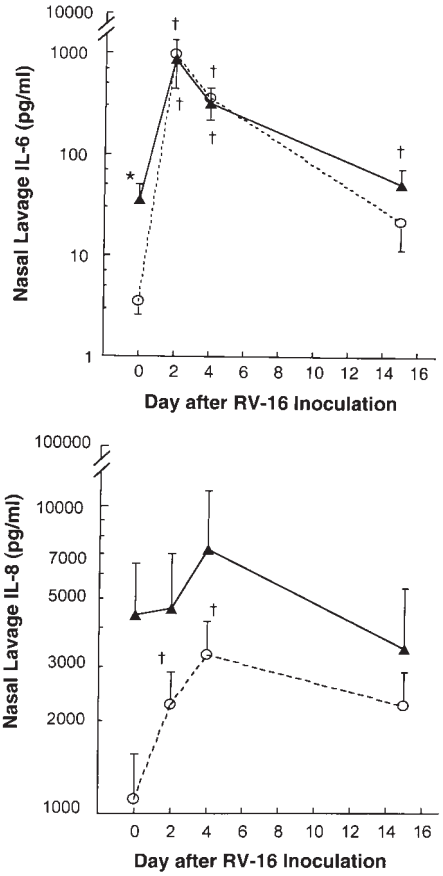

Figure 4. Nasal lavage IL-6 (*top panel*) and IL-8 (*bottom panel*) concentrations as mean ± SEM in healthy (*open circles*) and in asthmatic (*closed triangles*) subjects inoculated with RV-16 on Days 0 and 1. Asthmatic subjects had higher nasal IL-6 than did healthy subjects at baseline (*top panel*, *$p = 0.009$), and both groups had increased nasal IL-6 during the colds as compared with baseline (*top panel*, †$p \leq 0.02$). Only healthy subjects had a significant increase in IL-8 during the cold compared with baseline (*bottom panel*, †$p \leq 0.02$).

At baseline, the mean sputum neutrophil percentage in both groups was 41.3% (18.5 to 90.5%) and on Days 2 and 4 the mean increased to 49.4% (25.5 to 82.9%, $p = 0.005$). Eosinophils were more common in induced sputum from asthmatic subjects at baseline, but they did not increase after RV inoculation. IL-6 concentrations rose similarly in induced sputum samples in both groups (Figure 6, *top panel*), and IL-8 levels did not change. Interleukin-11 was not detected in induced sputum from any subject.

Asthmatic Who Experienced an Exacerbation

The single asthmatic subject who developed an exacerbation (Subject 21) requiring oral corticosteroid treatment on the fifth day after RV inoculation did not complete the protocol. This subject declined to use inhaled corticosteroid and required tapering doses of prednisone over 3 wk before recovering fully. She was the oldest subject (56 yr of age), but review of her baseline characteristics, cold symptoms, changes in pulmonary function, and nasal lavage and induced sputum samples revealed no notable differences from the other asthmatic subjects through the fourth postinoculation day.

DISCUSSION

In general, we found that the clinical, physiological, and inflammatory responses to nasal inoculation with RV-16 of healthy and asthmatic subjects were more similar than different. All subjects were seronegative for the strain of rhinovirus used, the inoculation was uniformly successful in causing infection, and the mean severity of the cold symptoms (Jackson's criteria) was nearly identical in the two groups. Only small changes were noted in pulmonary function and in inflammatory markers in induced sputum samples. These changes were not accompanied by clinically important events. Healthy subjects experienced an increase in methacholine reactivity during the cold, but no increase in lower airway symptoms, and no changes in PEF or in spirometry values. Likewise, the asthmatic subjects experienced a small increase in lower airway symptoms after the RV-16 inoculation, but they did not increase their β_2-agonist use, nor did they develop changes in pulmonary function.

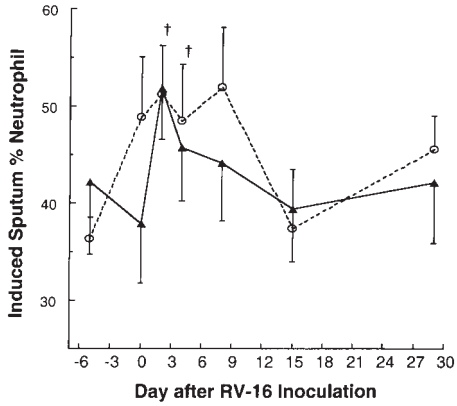

Figure 5. Induced sputum neutrophils as percent of nonsquamous cells (mean ± SEM) in healthy (*open circles*) and in asthmatic (*closed triangles*) subjects inoculated with RV-16 on Days 0 and 1. Taken together the two groups had a significant increase in percentage of neutrophils on Days 2 and 4 compared with baseline values ($^{\dagger}p \leq 0.01$). There was no significant difference between groups.

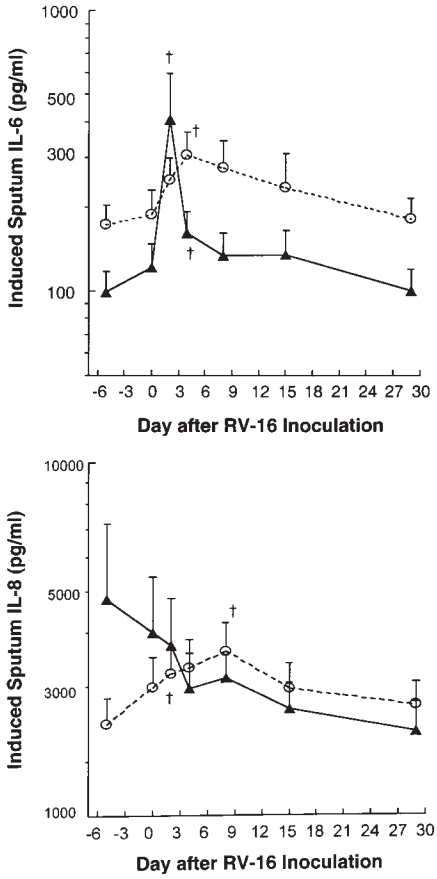

Figure 6. Induced sputum IL-6 (*top panel*) and IL-8 (*bottom panel*) concentrations (mean ± SEM) in healthy (*open circles*) and in asthmatic (*closed triangles*) subjects inoculated with RV-16 on Days 0 and 1. In both groups IL-6 increased significantly on Days 2 and 4 compared with baseline (*top panel*, $^{\dagger}p \leq 0.02$). In healthy subjects, IL-8 increased on Days 2 and 8 compared with baseline (*bottom panel*, $^{\dagger}p = 0.03$). There was no significant difference between groups for either cytokine.

Taken together, our study of the upper and lower airway responses to rhinovirus in healthy and asthmatic subjects largely confirms what has been established: that rhinovirus infection of the upper airway causes inflammation of both the upper and lower airways (15, 20, 21). Our findings differed from some (15, 21, 34, 35), but not all, previous studies (8), in that we found rhinovirus infection not to lead to an increase in bronchial reactivity to methacholine in asthmatics, although it did so in healthy subjects. A possible explanation for this difference between our findings and those of Grunberg and colleagues (21, 35) who did find rhinovirus inoculation to increase bronchial reactivity in asthmatics, is that they administered rhinovirus by aerosol as well as by direct instillation into the nose, perhaps increasing the intensity of infection of the lower airways. Moreover, Grunberg and colleagues used histamine to assess bronchial reactivity, which may be more sensitive than methacholine to detect changes during RV-16 colds (10, 11). Grunberg and colleagues (20) found no significant change in the cellular markers of inflammation in induced sputum samples obtained after inoculation in asthmatics, except for an increase in the proportion of neutrophils that stained positively for intracellular IL-8, whereas we found a small increase in the percentage of neutrophils in the asthmatic group, and in IL-8 in the healthy group.

Examining individual data from asthmatic subjects also provided no insight in the possible mechanism of rhinovirus-induced asthma exacerbation, if we consider exacerbation as the worsening of asthma symptoms requiring a significant increase in the rescue use of albuterol or the initiation of inhaled or oral corticosteroid treatment. The only subject who developed an asthma exacerbation after RV-16 inoculation (Subject 21) had no distinguishing feature from the other asthmatic subjects in terms of pulmonary function or inflammatory markers that could indicate a possible mechanism to explain why some experimentally RV-infected asthmatics develop asthma exacerbations. Examining the data from the subjects who completed the study, the only asthmatic who had both a $> 15\%$ decrease in FEV_1 and a > 1 doubling dose decrease in PC_{20} (Subject 20) had no cold symptoms, no change in asthma symptoms, and the changes in his nasal lavage and sputum samples resembled the median changes for the asthmatic group. The two asthmatic subjects with the most intense cold symptoms (Subjects 16 and 17) had the highest numbers of neutrophils and eosinophils and the highest concentrations of IL-8 in their nasal lavage fluid. This finding echoes those of Teran and colleagues (7), who reported a correlation between nasal IL-8 and cold symptoms in asthmatic children with community-acquired viral URIs, and those of Grunberg and colleagues (20), who also reported the same correlation in asthmatic subjects inoculated with RV-16. Interestingly, the nasal lavage samples from these two subjects with the most severe colds were also the only samples in which we could detect interleukin-5, RANTES, and interferon-γ, suggesting concurrent activation of lymphocytes with both the Th1- and Th2-like phenotypes. The nasal lavage fluid from these two subjects also showed the highest concentrations of IL-6 and IL-8 in the asthmatic group; and some of the highest increases in neutrophils among all subjects.

But searching intensely for evidence of mild inflammation in the lower airways of some asthmatics infected with rhinovirus 16 misdirects attention from our study's main finding: that even in asthmatic subjects, unequivocal infection of the upper airway with rhinovirus does not necessarily cause worsening of asthma. This is surprising because, as mentioned earlier, epidemiologic studies have shown rhinovirus common colds to be

the most common cause of asthma exacerbations. Our experience of having one of 11 rhinovirus-inoculated asthmatic subjects develop a clinically significant exacerbation resembles the experience of others (8, 15, 20, 34). Collating these results, three (5%) of 64 asthmatic subjects inoculated with rhinovirus developed exacerbations severe enough to require corticosteroid treatment or removal from the study.

Our own reasoning in attempting to reconcile the infrequency of exacerbations in asthmatics intentionally infected with rhinovirus with the strength of the association of rhinovirus infection with asthma exacerbations in epidemiologic studies runs along the following lines: first, the likelihood of developing an exacerbation may be a function of the severity of asthma, and all subjects in the reported studies had mild disease. Second, rhinoviruses may differ in their potency in provoking an exacerbation, and a limited number of the more than 100 different serotypes of rhinovirus have been used to inoculate asthmatic subjects. Some of the strains used, like ours, have doubtless undergone several passages, with a possible decrease in virulence compared with their native ancestors (36). This possibility is suggested by our failure to find interleukin-11 in the nasal lavage of any subject, whereas Einarsson and colleagues (23) have reported production of this cytokine to be a common feature of natural common colds accompanied by wheezing in asthmatic children. A third possibility is that there may be some interaction between the mechanisms of inflammatory responses to allergen inhalation and the mechanisms of inflammatory responses to viral infection. Supporting this possibility is the work of Anderson and Coyle (37), who demonstrated with a transgenic murine model that the response of CD8 lymphocytes, the cells thought to be critical in the defense against viral infection, to a virus glycoprotein can be switched from a Th1 (IFN-γ production and cytolytic activity) to a Th2 response (IL-5 production and loss of cytolytic activity) *in vivo* and *in vitro* through modulation with IL-4. It thus may be that rhinovirus infection at a time of ongoing exposure to sensitizing allergens triggers an exaggeration of the mechanisms of allergic inflammation, precipitating severe rhinitis or exacerbation of asthma. This theory may account for the rise in the levels of IL-5 and RANTES in the nasal lavage from our two asthmatic subjects with the most severe symptomatic colds. A final possibility of this incomplete list is that the requirement for the absence of neutralizing antibody could have excluded the very subjects most likely to develop an exacerbation. In this regard, Bardin and colleagues (14) have shown that rhinovirus-induced colds are more severe in allergic rhinitics than in healthy subjects who have previously been exposed to the same RV as shown by the presence of specific serum-neutralizing antibodies.

We regard as an important task the examination of possible differences among asthmatic subjects who experience asthma exacerbations during colds or among strains of rhinovirus involved in those exacerbations. Like others, we have shown that experimental infection with rhinovirus 16 is not necessarily sufficient to provoke an exacerbation in subjects with mild asthma. The development of effective strategies for prevention or treatment of asthma exacerbations associated with viral upper respiratory infection will require definition of the characteristics of the viruses and/or of the infected persons in whom an exacerbation of asthma occurs.

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