Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 cold

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Background: Despite the strong association of asthma exacerbations with rhinovirus (RV) infection, inoculation of asthmatic subjects with RV only causes small changes in lower airway function, suggesting that RV infection is not itself sufficient to provoke asthma exacerbations.
Objective: Our purpose was to test whether allergic inflammation increases the airway response to RV infection.
Methods: We compared the severity of RV type 16–induced colds in 2 groups of 10 subjects with allergic rhinitis. One group received 3 nasal challenges with allergen and the other received challenges with placebo over the week before nasal inoculation with RV type 16 (4000 tissue culture infective dose 50% per subject). Subjects kept symptom diaries and were assessed with spirometry, methacholine challenge, nasal lavage, and sputum induction on days 2, 4, 7, 10, 15, and 30 after inoculation.
Results: The 2 groups developed equal rates of infection (90%), similar cold symptoms (Jackson score median [interquartile range], 11 [6-33] vs 20.5 [6-42] for allergen and placebo groups respectively, $P = .54$), and similar changes in cellular profile and in IL-6 and IL-8 concentrations in nasal lavage fluid and induced sputum after RV inoculation. The incubation period was significantly longer in the allergen group (2.5 [1-5.5] vs 1 [1-1] day, $P = .03$) and the duration of cold symptoms was shorter (5 [4-7] vs 8.5 [6-10] days, $P = .008$). We also found an inverse correlation between the percent of eosinophils in nasal lavage fluid before inoculation and the severity of cold symptoms ($r = –0.58$, $P = .008$).
Conclusion: In subjects with allergic rhinitis, augmented nasal allergic inflammation before inoculation with RV type 16 does not worsen the severity of cold symptoms but delays their onset and shortens their duration. (J Allergy Clin Immunol 2000;105:923-32.)

Key words: *Rhinovirus, nasal allergen challenge, common colds, allergic rhinitis*

Most asthma exacerbations are associated with infection with a respiratory virus, particularly rhinovirus (RV).[1,2] However, when asthmatic subjects are assessed during naturally acquired or experimentally induced common colds, only small and inconsistent changes occur in airway function; clinical worsening of asthma rarely occurs.[3,4] For example, some investigators have reported modest but significant increases in bronchial responsiveness to histamine,[5-10] methacholine,[7,11] and allergen challenge.[6,7,12] Others have reported increases in lymphocyte and eosinophil numbers in bronchial mucosa.[8,9] Other investigators, however, have failed to detect changes in bronchial reactivity during experimental colds.[13-16] In our own comparison of the severity of upper and lower airway changes in healthy and asthmatic subjects inoculated with rhinovirus serotype 16 (RV-16), we found the severity of cold symptoms and the relative changes in pulmonary function to be highly similar.[4] Like most other studies of experimental colds in asthmatic subjects,[8,10,11,13,17] we found only small increases in asthmatic symptoms and no significant increase in the rescue use of bronchodilators. We therefore speculated that some other factor or mechanism must be operative for RV infection to cause worsening of asthma.

Possible candidates for this other factor or mechanism are differences in the virulence of cold viruses or differences in host immune responses. That virulence may differ among cold viruses is suggested by studies showing greater changes in lower airway function after inoculation with RV-16[6,8,10] than with RV-2,[18] RV-39,[13,16] RV Hanks,[13,14] or attenuated influenza A virus.[15] These differences, however, cannot account for the great variability of upper and lower airway responses among subjects within a single study, where subjects are inoculated by the same methods with the same strain of virus. This calls attention to possible differences in host factors shaping the response to viral infection.

Possible host factors that could influence the response to viral infection include immunity resulting from previous exposure to the same or a similar virus,[19] HLA type, and modulation by cytokines. Animal and clinical studies make the last possible factor appear especially likely. With use of transgenic mice engineered so that most of their CD8 cells respond to a virus glycoprotein, Coyle et al[20] demonstrated that the CD8 cell response to that gly-

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Abbreviations used
EIA: Enzyme immunoassay
mRNA: Messenger RNA
NO: Nitric oxide
PC_{20}: Provocative concentration causing a 20% decrease in FEV_1
RSV: Respiratory syncytial virus
RV: Rhinovirus
TCID50: Tissue culture infective dose 50%
T_H: T helper lymphocyte

coprotein could be switched from a T helper lymphocyte (T_H) type 1 (T_H1 to a T_H2 pattern if the CD8 cells were exposed to mediators of allergic inflammation (exogenous IL-4 in vitro or allergen challenge in vivo). These findings led to the hypothesis that active allergic inflammation can switch the response to a respiratory virus from a T_H1 to a T_H2 pattern, so that infection results in an amplification of allergic airway inflammation.[21] Another mechanism by which allergic inflammation could worsen rhinovirus colds is by increasing expression of intercellular adhesion molecule-1, the receptor for rhinoviruses,[22] on nasal epithelial cells[23] and fibroblasts.[24]

Clinical studies of subjects with allergic asthma or rhinitis have provided inconsistent support for this hypothesis. Some studies have reported that experimental colds cause greater increases in nonspecific airway reactivity[8,25] and produce more severe cold symptoms[26] in atopic than in healthy nonatopic subjects, whereas others have found no differences.[14-16] Noting that these studies of atopic subjects were done out of the relevant pollen season[8,9,14-16,25,27] and that in Coyle's animal experiments[20] the switch of virus antigen response from a T_H1 to a T_H2 pattern occurred when allergic inflammation was induced just before virus antigen exposure, we hypothesized that inducing allergic inflammation just before RV inoculation would amplify the local response to infection in atopic subjects. We have first tested this hypothesis in the nasal airway because the nose is the principal site of rhinovirus infection and because we thought that any great amplification of the nasal response would be tolerated more safely than a great amplification of the response of the lower airway.

METHODS

Study subjects

Our protocol was approved by the Committee on Human Research and by the Biosafety Committee of the University of California San Francisco. Subjects were recruited through advertisement and provided written informed consent to participate in the study. Inclusion criteria were age between 18 and 55 years, a history of allergic rhinitis with positive allergen skin tests, and serum neutralizing antibody titer for RV-16 ≤2. Exclusion criteria were smoking history within 1 year or >5 pack-years lifetime, cold symptoms or use of topical or systemic corticosteroids in the previous 4 weeks, asthma requiring more than intermittent bronchodilator treatment, allergen immunotherapy, regular use of allergic medications, use of anti-inflammatory medications, and respiratory disease other than allergic rhinitis and mild intermittent asthma. No medication was allowed during the study except for use of inhaled β_2-agonists and acetaminophen as needed. Baseline characteristics of study subjects were similar in the 2 groups (Table I).

Study design

Baseline measurements were obtained during the first week of this 6-week, double-blinded, randomized, placebo-controlled study (Fig 1). In the second week subjects were randomized into 2 groups: one group to receive 3 nasal allergen challenges (allergen group) and the other to receive 3 nasal placebo challenges (placebo group). Nasal challenges were performed on alternate days (days –7, –5, and –3) in a double-blinded fashion. Vials containing allergen were prepared and blinded by an investigator not involved with the visit procedures. Placebo was calcium- and magnesium-free PBS (University of California, San Francisco, Cell Culture Facility). Three days after the last nasal challenge, all subjects were inoculated with RV-16 on 2 consecutive days (days 0 and 1).

In a pilot study of 5 subjects we confirmed that nasal symptoms returned to baseline 2 days after the last nasal allergen challenge. Because our main outcome was cold symptoms, we waited 72 hours after the last challenge to ensure that all nasal symptoms had regressed to baseline before RV-16 inoculation.

All subjects recorded the severity of cold and chest symptoms in diaries throughout the study. On day 28 serologic studies for RV-16 were repeated to verify seroconversion. Assessment of airway disease was made by symptom diary, peak flow monitoring, spirometry, PC_{20}, nasal lavage and sputum induction.

Symptom assessment and peak flow monitoring

Subjects kept diaries recording common cold and chest symptoms each evening throughout the study. Cold symptom severity was assessed with the validated Jackson cold score[4,28] in which subjects graded from 0 to 3 (absent, mild, moderate, or severe) 8 symptoms: nasal discharge, nasal congestion, sneezing, sore throat, cough, headache, malaise, and fever/chills. The Jackson cold score was calculated as the sum of the grades for all symptoms during 6 days after RV-16 inoculation (days 1 to 6), minus the sum during the baseline week (days –13 to –8). To compare these symptoms with those caused by the allergen challenges, a similar score was calculated for the week of nasal challenges (sum of days –7 to –2 minus the sum of days –13 to –8) and referred to as nasal challenge symptom score. For the clinical diagnosis of a cold, a subject had to meet either of the following 2 criteria: (1) a Jackson cold score >13 combined with either increased rhinorrhea for ≥3 days after RV inoculation or the subject's perception of a cold or (2) if the cold score was ≤13, the combination of the perception of a cold and increased rhinorrhea for ≥3 days.[28] Infection by RV-16 was documented by either recovery of RV-16 from nasal lavage or seroconversion of neutralizing antibodies to RV-16 (≥4-fold rise in titer). Incubation period was measured in days from RV-16 inoculation (day 0) to the onset of cold symptoms. Because many subjects, particularly in the allergen group, had longer incubation periods than expected, we adjusted the Jackson score for the incubation period for each subject. The adjusted score was calculated as the sum of all grades for cold symptoms over the 6 days after their first appearance minus the sum over the baseline period (days –13 to –8). Time to peak of symptoms was measured in days from RV-16 inoculation (day 0) to the day of maximum total daily cold symptom score.

Chest symptoms were assessed with a validated asthma symptom score[29] in which the subjects graded on an ordinal scale from 0(none) to 10(extremely severe) 5 symptoms of asthma: shortness of breath, chest tightness, wheezing, cough, and sputum/phlegm. Subjects were given an Air-Watch peak flow meter (ENACT Health Management Systems, Mountain View, Calif) and instructed to perform peak flow maneuvers in triplicate twice daily.[4]

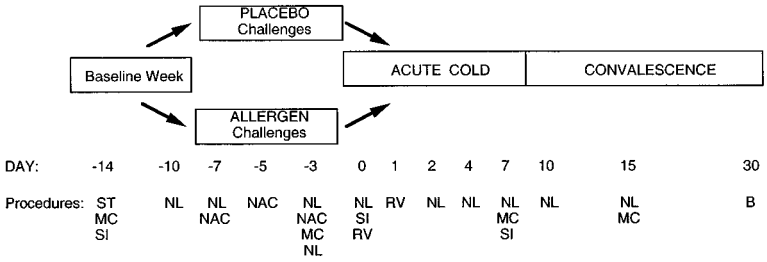

FIG 1. Sequence of study visits and procedures in the order they were performed. Subjects kept a symptom diary and performed peak flow measurements daily. During the initial week subjects underwent skin prick test and titration *(ST),* spirometry *(S),* methacholine challenge *(MC),* sputum induction *(SI),* and nasal lavage *(NL);* these were repeated throughout the study as shown. In the second week subjects received 3 nasal challenges *(NAC)* with either allergen or placebo. The second nasal lavage on day –3 was performed 1 hour after the last dose of the nasal challenge. In the third week subjects were inoculated with RV-16 on days 0 and 1. A blood draw *(B)* on day 30 assessed seroconversion to RV-16.

TABLE I. Baseline clinical characteristics of subjects

Group	Age (y)	Sex	Race	FEV_1 (L)	FEV_1 (% predicted)	PC_{20} (mg/mL)	RV-16 Ab titer	Skin test sensitivity	Dose of allergen*
Allergen									
1	25	F	C	2.8	90	108	0	G, C	625
2	36	M	C	3.9	98	125	0	G, DF, DP	125
3	26	M	C	5.8	129	38	0	C, DF, DP, T, G	3125
4	23	F	C	3.5	100	85	0	DP, DF	5
5	33	F	B	3.2	110	8	2	T, DF	5
6	22	M	H	3.9	91	5	0	G, DF, DP	625
7	22	M	C	5.9	118	87	1	DP, C, DF, T, G	125
8	48	F	C	2.5	96	70	1	G	125
9	28	F	C	3.9	111	104	0	DP, C, DF, T, G	5
10	32	M	H	4.1	103	164	1	DP, DF	125
Median	27	5M		3.8	102	86			
(IQR)	(23-34)			(3.1-4.6)	(95-113)	(31-112)			
Placebo									
11	26	M	C	5.3	96	85	0	DP, C, DF, T, G	—
12	29	M	H	3.5	90	279	0	C, DF, DP	—
13	48	F	B	2.7	117	6	0	C, DP, T, G	—
14	44	F	C	2.9	94	90	0	T, G	—
15	37	F	C	3.2	119	0.3	1	DP, C, DF	—
16	25	M	H	4.6	98	6	0	DP, C, DF, G	—
17	32	M	C	4.5	102	1	0	DP, C, DF	—
18	40	F	C	3.1	94	5	0	DP, C, DF, T, G	—
19	26	M	A	4.4	102	172	1	G, C, T	—
20	40	M	C	3.7	95	15	0	G	—
Median	34	6M		3.6	97	11			
(IQR)	(26-41)			(3.1-4.5)	(94-106)	(4-110)			

PC_{20}, Provocative concentration of methacholine causing a 20% drop from baseline FEV_1; *RV-16 Ab titer,* Titer is the reciprocal of the highest dilution of serum causing neutralization of 100 tissue culture infective dose 50% (TCID50) of RV-16; *titer = 0,* failure of undiluted serum to neutralize RV-16; *skin test sensitivity,* assessed for rye grass (G), cat (C), dust mites (DF and DP, see Methods), and birch tree (T) and the allergen that elicited the largest skin reaction is listed first (chosen for nasal challenges in the allergen group); *F,* female; *C,* Caucasian; *M,* male; *B,* black; *H,* Hispanic; *IQR,* interquartile range; *A,* Asian. *Expressed as the reciprocal of the last dilution administered in the first nasal challenge. Subjects were studied out of their pollen season and they were not living with pets. There were no significant differences between groups.

Allergen skin tests

Allergy skin prick tests were performed in duplicate with plastic needles (Morrow Brown Allergy Diagnostics, Oakhurst, NJ) with use of 5 extracts: *Dermatophagoides pteronyssinus* (10,000 BAU/mL), *Dermatophagoides farinae* (10,000 BAU/mL), cat hair (10,000 BAU/mL), rye grass (2% wt/vol), birch tree (2% wt/vol), and negative control (Bayer Corporation, Elkhart, Ind).[4] Positive control was histamine base 1 mg/mL (Histatrol, Center Lab, Port Washington, NY). A reaction was positive if it elicited ≥3 mm wheal with erythema at 15 minutes. The allergen producing the largest wheal was used for the skin prick test titration in duplicate of 5-fold dilutions in calcium- and magnesium-free PBS from 1:5 to 1:78,125.

Nasal challenges

Nasal challenges were delivered with Atomizer #15 DeVilbiss (Somerset, Pa), which produces coarse droplets unlikely to be inhaled into the lungs. A 3-mL tube (E & K Scientific Products,

Campbell, Calif) was used to hold 2 mL of the challenging solution. The atomizer was connected to a dosimeter (DSM-2, S&M Instrument, Doylestown, Pa) and actuation was set at 10 psi of pressure, 0.1 second of duration, and 2 seconds of standby time. For each dilution, 3 actuations were administered into each nostril (0.04 mL/actuation) during resting inspiration with the subject's head elevated 30 degrees. After 15 minutes the subject graded nasal congestion and nasal discharge from 0 to 3 (absent, mild, moderate, and severe) and number of sneezes (none, 1-3, 4-6, ≥7 sneezes). A nasal symptom score was calculated by summing these grades (range 0 to 9). Nasal inspiratory peak flow (Youlten peak nasal inspiratory flow meter, Clement Clarke International) and spirometry were also measured.

Challenging dilutions started with diluent nasal challenge (calcium- and magnesium-free PBS) followed by either increasing 5-fold concentrations of allergen or repeat diluent challenges according to randomization. Challenges were continued until either the subject's nasal inspiratory peak flow dropped ≥50%, the subject had a nasal symptom score of ≥6, or the last dilution (1:5 or diluent) had been administered 3 times.

On the first nasal challenge (day –7), the first allergen dilution given after the initial diluent challenge was 5-fold more diluted than the weakest dilution causing a positive reaction in the allergen skin prick test titration. For the second and third nasal challenges (days –5 and –3), the first allergen dilution was 1:5 (ie, one step below) of the last dilution administered in the first nasal challenge. The last dilution of allergen given in the second and third nasal allergen challenges were within one 5-fold dilution for 95% of those challenges.

Pulmonary physiology tests

Spirometry was performed with a dry-rolling seal spirometer (Ohio 840), and methacholine challenge (0.078 to 80 mg/mL) was performed by the dosimeter technique as previously described.[4] For subjects who did not drop ≥20% at 80 mg/mL of methacholine, PC_{20} was extrapolated from the slope of the drop in FEV_1 as previously described.[30]

Rhinovirus procedures

The strain of RV-16 we used was generously provided by William Busse, MD, and Elliot Dick, PhD (University of Wisconsin, Madison, Wis). A passage 3 was safety tested for human use[31,32] and titrated to 1000 TCID50 per milliliter.[4] Subjects were inoculated with RV-16 on 2 consecutive days (days 0 and 1). On each day, 0.5 mL of a 1000 TCID50 per milliliter suspension was instilled with a transfer pipette into each nostril, followed by spraying 0.5 mL into each nostril with atomizer 15 (4 actuations of 0.4 seconds at 10 psi). Thus each subject was inoculated with 4000 TCID50 of RV-16.

Nasal lavage titer was expressed as TCID50 and serum neutralizing anti-RV-16 antibodies as the reciprocal of the highest dilution to cause neutralization of the RV-16 challenge.[4]

Nasal lavage and sputum induction after RV-16 inoculation

Nasal lavage was performed by consecutively instilling 5 mL of warmed (33°C) calcium- and magnesium-free PBS solution into each nostril and having the subject blow his or her nose into a plastic cup after a 20-second dwell time. Sputum induction was performed by nebulization of 3.0% saline solution for 12 minutes.[4] Sputum and nasal lavage samples were processed as previously described[4] to obtain total cell count, differential count of all cells for nasal lavage and of nonsquamous cells for induced sputum, and measurement of cytokines in the supernatant.

Quantification of cytokines

IL-4, IL-5, IL-6, IL-8, IL-12, and IFN-γ were measured by enzyme immunoassay (EIA) according to the manufacturer's instructions (Quantikine kits from R&D Systems, Minneapolis, Minn). The reported sensitivity of the assays were, respectively, 0.13, 3.0, 0.7, 10.0, 0.6, and 3.0 pg/mL.

Statistical analysis

Data were analyzed with StatView (BrainPower, Calabasas, Calif) and Stata (Stata, College Station, Tex) softwares. Values are expressed as median and interquartile range. Repeated measures were analyzed with the Friedman test, and when significant, between-group comparisons were made with the Mann-Whitney rank sum test and paired comparisons within group with the Wilcoxon signed-rank test. Proportions were compared with the Fisher exact test and correlations were analyzed with the Spearman rank-order correlation test. Results were considered statistically significant when P values were $<.05$. Data from all 20 subjects were analyzed by intention to treat.

RESULTS

Nasal allergen challenges

Nasal allergen challenges were successful in increasing symptoms and allergic inflammation. Subjects in the allergen group had significantly more nasal symptoms than did subjects in the placebo group during the week of nasal challenges ($P = .001$, Table II and Fig 2). On day –3, 1 hour after the third nasal challenge, the median percent eosinophil in nasal lavage fluid was 17.6% (interquartile range 4.4% to 41%) in the allergen group and 1.4% (0.2% to 10%) in the placebo group ($P = .015$). The median nasal symptom scores and percent eosinophils in nasal lavage returned to baseline in all subjects within 3 days (ie, by day 0 before the nasal inoculation with RV-16) (Figs 2 and 3, *B*).

Common cold symptoms

In both groups 90% of the subjects were infected by RV-16 as demonstrated by nasal lavage culture or seroconversion, and 80% had clinical colds according to Jackson criteria. Nasal lavage titers of RV-16 were similar in both groups (Table II).

The difference in severity of the common cold symptoms between the 2 groups did not reach statistical significance (median Jackson score of 11 in the allergen group vs 20.5 for the placebo group, $P = .54$) and was even more similar when calculated over the 6 days after the first appearance of cold symptoms, that is, adjusted for the incubation period (20 vs 19.5, $P = .87$, see Table II and Fig 2).

Unexpectedly, there was a significant delay in the onset of common cold symptoms in the allergen group. The median interval between nasal inoculation and the first perception of cold symptoms (incubation period) was 2.5 days in the allergen group and 1 day in the placebo group ($P = .03$). Furthermore, the duration of cold symptoms, from the day of first perception of cold symptoms to the day when total daily cold symptom score returned to baseline (score from day –1), was shorter in the allergen group than in the placebo group (5 vs 8.5 days, $P = .008$, Table II).

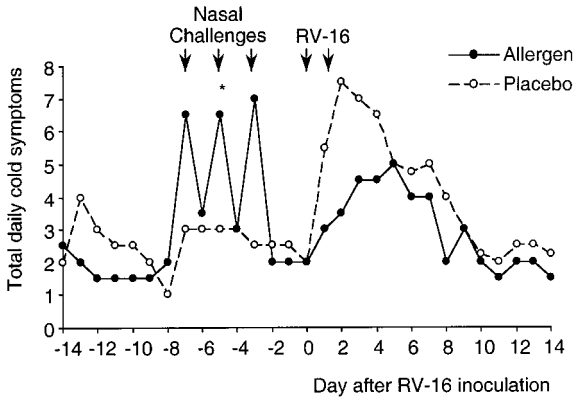

FIG 2. Median total daily Jackson cold symptoms in allergic rhinitis subjects who underwent nasal allergen *(closed circles)* or placebo *(open circles)* challenges on days –7, –5, and –3 and nasal inoculation with RV-16 on days 0 and 1. The allergen group had significantly greater nasal symptoms than the placebo group did (*asterisk, P* = .001) during the week of nasal challenges. After nasal challlenges, symptoms returned to baseline on day 0 before RV-16 inoculation. The increases in cold symptoms after RV-16 inoculation in the 2 groups did not differ significantly.

TABLE II. Common cold variables in subjects with allergic rhinitis primed with allergen or placebo nasal challenges before inoculation with RV-16

Subjects	Nasal challenge symptom score	Cold symptom score	Duration of increased rhinorrhea during cold days	Perceived a cold	RV-16 titer in nasal lavage fluid (10^x/mL)*	Incubation period (d)	Cold symptom score adjusted for incubation period	Time from inoculation to peak of symptoms (d)	Duration of cold (d)	Post-infection Ab titer to RV-16†
Allergen										
1	9	42	5	Yes	2	1	42	3	7	16
2	32	9	3	Yes	5.5	6	27	8	4	0
3	11	37	3	Yes	5	1	37	1	8	2
4	11	11	4	Yes	5	6	25	7	5	16
5	11	10	6	Yes	7	4	15	6	6	16
6	7	–5	—	No	0	—	–5	—	—	8
7	29	13	3	Yes	4	1	13	2	4	16
8	18	–3	—	No	3.5	—	–3	—	—	1
9	10	32	2	Yes	4	2	31	3	5	1
10	13	11	3	Yes	0	3	11	3	4	2
Median	11	11	3	80%	4	2.5	20	3	5	50%
(IQR)	(10-21)	(6-33)	(3-5)		(1.5-5)	(1-5.5)	(8-33)	(2-7)	(4-7)	
Placebo										
11	–1	41	9	Yes	4.5	1	41	3	10	2
12	15	29	0	Yes	4.5	2	27	4	6	0
13	–13	45	9	Yes	7.5	1	45	2	10	64
14	–2	6	—	No	0	—	6	—	—	0
15	–3	–8	—	No	0	—	–8	—	—	16
16	3	12	4	Yes	3	1	12	1	8	4
17	1	47	4	Yes	6	1	47	2	7	8
18	9	29	9	Yes	4	1	29	2	9	4
19	4	11	4	Yes	3	1	11	2	6	8
20	–11	5	7	Yes	3.5	1	8	3	9	16
Mean	0	20.5	5.5	80%	3.75	1	19.5	1.7	8.5	70%
(IQR)	(–5-5)	(6-42)	(4-9)		(2-5)	(1-1)	(7.5-42)	(1-2)	(6-10)	
Statistical significance	*P* = .002	NS	*P* = .08	NS	NS	*P* = .03	NS	*P* = .09	*P* = .008	NS

Comparison between groups by Mann-Whitney rank sum test or Fisher exact tests for proportions. *NS,* Not significant.
*Titer in TCID50 obtained by culturing 0.1 mL of nasal lavage fluid in duplicate (see Methods).
†Titer is the reciprocal of the highest dilution of serum causing neutralization of 100 TCID50. Percentage indicates the proportion of seroconversion (4-fold or higher increase in titer).

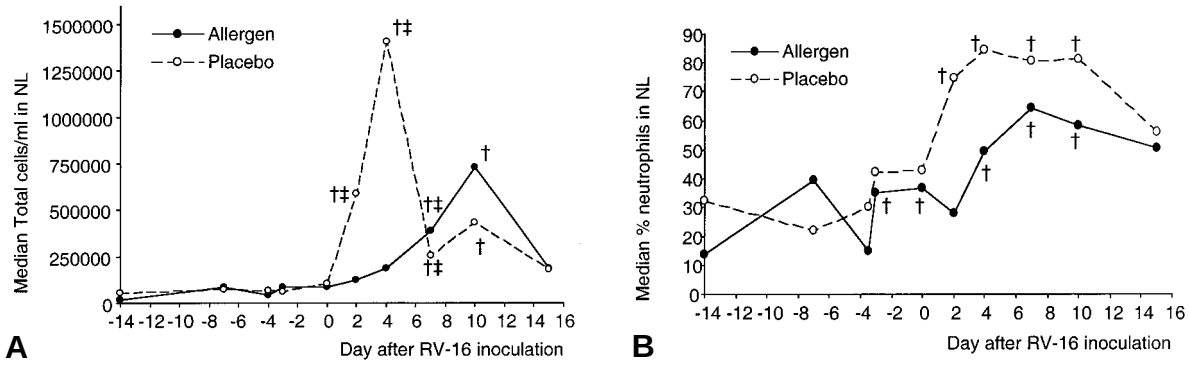

FIG 3. Median total cells per milliliter (**A**) and percentage of neutrophils (**B**) in nasal lavage *(NL)* from allergic rhinitic subjects at various time points. Both the allergen group *(closed circles)* and the placebo group *(open circles)* had similar and significant increases in the median total cells and percent neutrophils in nasal lavage fluid after RV-16 inoculation compared with baseline (day –14; *dagger, P* < .05) and with preinoculation (day 0; *double dagger, P* < .05) values, but the increases were delayed in the allergen group.

Nasal lavage and induced sputum during cold

Differential cell counts of nasal lavage fluid revealed the same changes in both groups, namely, a significant increase in total cells and percentage neutrophils during the common cold. These changes paralleled the changes in symptoms, that is, they were delayed in the allergen group but of similar magnitude in both groups (Table III and Fig 3). The percentage of eosinophils did not increase in either group during the cold.

Cytokine measurements in nasal lavage fluid showed increases in IL-8 and IL-6 concentrations during the common cold in both groups. Again, those changes were delayed in the allergen group but were of similar magnitude to those seen in the placebo group (Table III and Fig 4).

Unlike the nasal lavage results, induced sputum samples showed no marked changes in differential cell count of nonsquamous cells or in IL-6 and IL-8 concentrations between or within groups (data not shown).

Analyzing all subjects together, we found that the percent of eosinophils in nasal lavage fluid on day 0, just before RV-16 inoculation, correlated inversely with common cold symptoms, both Jackson score and Jackson score adjusted for incubation period ($r = –0.58$, $P = .008$ and $r = –0.65$, $P = .002$, respectively, Fig 5).

As other studies did, we also found significant correlations between cold symptoms (adjusted Jackson score) and neutrophils ($r = 0.71$, $P = .0004$), IL-6 ($r = 0.76$, $P = .0001$), and IL-8 ($r = 0.65$, $P = .002$) in nasal secretions summarized as area under the curve during the cold (days 0 to 15).

We used EIA kits to measure T_H1 (IFN-γ and IL-12) and T_H2 (IL-4 and IL-5) cytokines in nasal lavage and sputum samples. Very few nasal lavage and induced sputum samples had detectable levels of IL-5 and IFN-γ. IL-4 and IL-12 were assayed in the nasal lavage fluid from the subjects with the worst colds in each group (subjects 2, 5, 11, 13) and none was detected.

Pulmonary function

Serial spirometries showed a nonsignificant similar mild decrease in FEV_1 (4% decrease from baseline) on day 4 after inoculation of RV-16 in both groups. Reactivity to methacholine did not change significantly in either group (data not shown).

DISCUSSION

In this study, we found that priming the nasal mucosa with nasal allergen challenges before RV-16 inoculation did not increase the severity of cold symptoms but did delay their onset and shortened their duration. This was also true for the increases in neutrophils and in IL-6 and IL-8 in nasal lavage fluid. We also found an inverse correlation between the percentage of eosinophils in the nasal lavage just before RV-16 inoculation and the severity of the colds. We thus think it is possible that nasal allergic inflammation may attenuate RV-16 colds.

It might be argued that the preceding nasal allergen challenges increased the perception threshold for nasal symptoms of the subjects in the allergen group, which could explain the delay in the onset of reported cold symptoms. However, the delay in appearance of subjective symptoms was accompanied by a delay in appearance of signs (eg, purulent rhinorrhea) and of objective changes in nasal lavage (percent neutrophils, IL-6, and IL-8), corroborating a real delay in the onset of RV-16–induced nasal inflammation.

Given the greater-than-expected variability in symptom scores, we acknowledge that our sample size was small for detecting differences in cold symptoms between the 2 groups. However, we believe that our results provide sufficient evidence that priming the nasal mucosa with repeated allergen challenges before rhinovirus inoculation does not worsen cold symptoms. Cold symptoms were similar in both groups and, if anything, were milder in the allergen group. This group had a significantly shorter duration of cold symptoms. Our

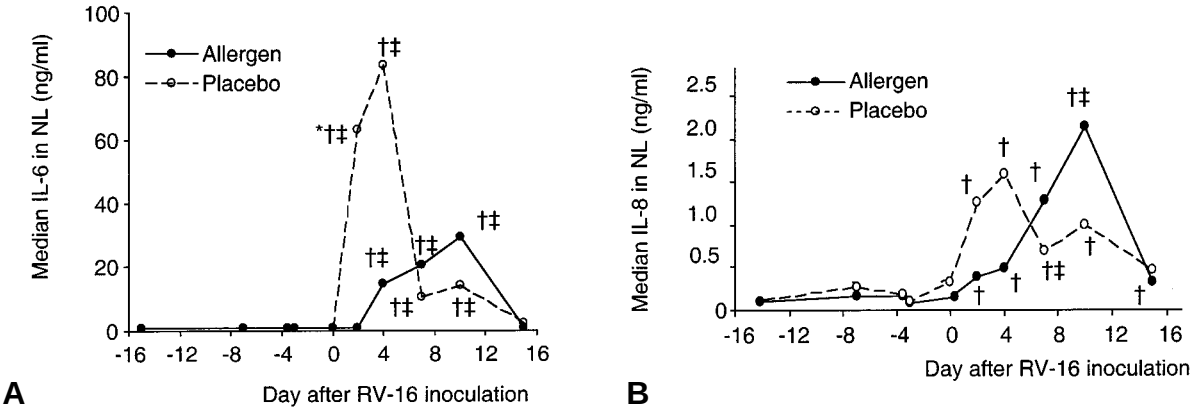

FIG 4. Median concentration of IL-6 (**A**) and IL-8 (**B**) in nasal lavage fluid *(NL)* of allergic rhinitic subjects primed with allergen (allergen group, *closed circles*) or placebo (placebo group, *open circles*) on days –7, –5, and –3 before RV-16 inoculation on days 0 and 1. IL-6 and IL-8 increased significantly in both groups after RV-16 inoculation compared with baseline (day –14; *dagger, P* < .05) and with preinoculation (day 0; *double dagger, P* < .05) levels. On day 2, nasal IL-6 was significantly lower in the allergen group than in the placebo group (*asterisk, P* < .05) because of the delayed rise in IL-6 in the allergen group. There was no significant difference in nasal IL-8 between the 2 groups.

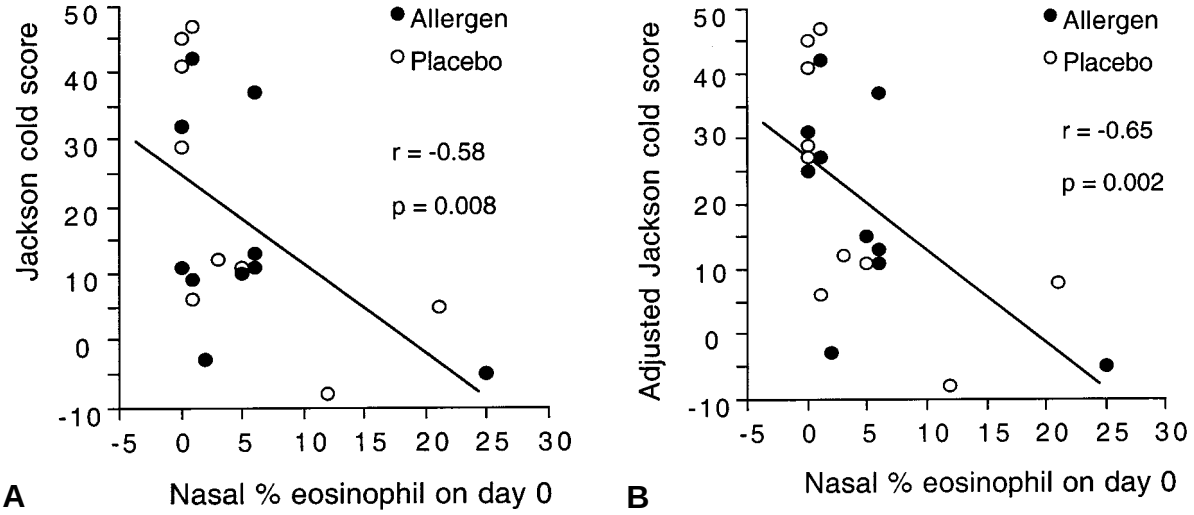

FIG 5. When all subjects from the allergen *(closed circles)* and placebo groups *(open circles)* were analyzed together, there was an inverse correlation between the median percent of eosinophils in nasal lavage fluid immediately before RV-16 inoculation (day 0) and the severity of cold symptoms assessed by the Jackson cold score (**A**, $r = –0.58$, $P = .008$). This correlation was even stronger when the Jackson cold score was adjusted for the incubation period (**B**, $r = –0.65$, $P = .002$) and persisted even after the 3 subjects with the highest values for percent eosinophils were removed ($r = –0.47$, $P = .05$).

results speak strongly against our initial hypothesis that allergen exposure before rhinovirus infection worsens cold symptoms.

Comparing our results with those of previous studies reveals both similarities and differences. Our findings resemble those of previous studies showing RV-16 inoculation to induce upper but not lower airway symptoms.[14-16,25,27] Our inability to find an increase in bronchial reactivity during experimental RV colds in subjects with allergic rhinitis, unlike other investigators,[6-8, 10, 11, 25] could be due to our use of methacholine as the provocative agent. When histamine[7,8,10,25] rather than methacholine[7,11,33] is used, changes in bronchial reactivity during RV-16–induced colds have more consistently been demonstrated. Our results also seem to differ from those of a previous study that reported more severe cold symptoms in atopic than in nonatopic subjects inoculated with RV-16.[26] That difference was found, however, only among atopic and nonatopic subjects who were seropositive for RV-16, and not among seronegative subjects who developed colds of similar severity regardless of atopic status.

TABLE III. Results of nasal lavage variables in subjects with allergic rhinitis

Variable	Group	Baseline*	Before third NAC†	After third NAC	Day 0	Day 2	Day 4	Day 7	Day 10	Day 15
Nasal lavage fluid										
Total cells	Allergen	0.2	0.5	0.9	0.9	1.3	1.9	3.9	7.3	1.9
($\times 10^5$/mL)		(0.1-1.0)	(0.3-0.8)	(0.6-1.3)	(0.6-1.7)‡	(0.8-2.4)‡	(0.4-23)‡	(1.6-39)‡§	(0.8-17)‡	(0.4-3.4)‡
	Placebo	0.6	0.8	0.6	1.1	5.9	14.1	2.6	4.3	1.8
		(0.4-2.2)	(0.2-1.4)	(0.2-2.1)	(0.5-1.7)	(0.9-162)‡§	(0.5-122)‡§	(0.2-33)‡§	(0.9-21)‡	(0.8-4.4.)
Eosinophils (%)	Allergen	0.6	1.9	17.6	3.6	2.5	3.3	1.1	2.3	2.1
		(0.4-2)	(0.7-20)	(4.4-41)‡	(0.5-6)	(0.3-6)	(0.5-6)	(0.5-3.8)	(0.2-4.6)	(0.3-12)
	Placebo	1.4	1.8	1.4	1.3	2.8	0.8	0.8	1.6	1
		(0.6-8)	(0.2-22)	(0.2-10)	(0-6.5)	(0.4-6)	(0.3-6.8)	(0.2-4)	(0.4-4.7)	(0.2-3.2)
Neutrophils (%)	Allergen	13.5	14.7	35	37	28	49	64	58	50.5
		(4.4-33)	(1-51)	(25-60)‡	(10-62)‡	(13-53)	(23-92)‡	(48-92)‡§	(44-86)‡	(15-67)
	Placebo	32.4	30.3	42	43	75	85	81	81	56
		(3-59)	(10-56)	(29-83)	(3-74)	(17-92)‡	(24-97)‡	(40-97)‡	(37-90)‡	(10-87)
IL-6 (pg/mL)	Allergen	0	0	0	0	0	14.8	20.7	29.3	0
		(0-0)	(0-0)	(0-0)	(0-1.6)	(0-46)	(0-91)‡§	(3-121)‡§	(0-115)‡§	(0-10)
	Placebo	0	0	0	0	63.3	83.6	10.6	14.1	2.5
		(0-0)	(0-3.6)	(0-4.4)	(0-10)	(6-522)‡§‖	(0-219)‡§	(4-74)‡§	(3-33)‡§	(0-6)
IL-8 (pg/mL)	Allergen	0.10	0.16	0.08	0.14	0.38	0.49	1.29	2.12	0.32
		(0-0.4)	(0-0.35)	(0-0.14)	(0.4-0.7)	(0.1-2.33)‡	(0.2-2.8)‡	(0.5-3.3)‡	(0.2-32)‡§	(0-8.2)‡
	Placebo	0.10	0.19	0.10	0.32	1.25	1.6	0.70	2.6	0.47
		(0-0.6)	(0-2.5)	(0-0.3)	(0-0.6)	(0.3-3.2)‡	(0.2-3.2)‡	(0.2-2.6)‡§	(0.6-5.4)‡	(0.3-0.8)‡

Median (interquartile range) of values at baseline* during the first week, before and after the third nasal challenge† on day –3, and during the 15 days after RV-16 inoculation. For all variables, repeated-measures Friedman test yielded $P < .002$, except for percent eosinophils in the placebo group ($P > .05$). Significant differences ($P < .05$) between groups on the same day‖ and within either group compared with baseline (day –14)‡ and with preinoculation values (day 0)§ are depicted.

The most intriguing finding in our study is that the severity of cold symptoms was not increased by preceding augmentation of nasal allergic inflammation because animal studies with similar study design have found greater lower airway inflammation in animals intratracheally challenged with allergen before respiratory syncytial virus (RSV) inoculation than in animals that received either intervention alone.[34] However, RSV causes marked cytopathic effects in epithelial cells in the lower airways, whereas RV causes little to no damage to epithelial cells[3] and it probably replicates preferentially in the upper rather than the lower airways because of its temperature sensitivity.[35] The reverse sequence, RV-16 inoculation followed by bronchial allergen challenge, has been done in atopic subjects and was shown to be associated with more severe reactions to allergen challenges such as a higher incidence of late-phase bronchial reactions, greater increases in bronchial reactivity, and greater release of histamine and TNF-α into the airway.[6,7,12] This shows that the interactions between allergen-induced and rhinovirus-induced inflammatory responses are complex. It is conceivable that the interactions between these inflammatory responses are different in the lower and upper airways, and it is still possible that in the lower airways allergic inflammation promotes RV infection or alters the response to RV. It is also possible that repeated low-dose allergen challenges (instead of the high-dose challenges used in our study) or a single allergen exposure before RV infection could amplify the response to subsequent RV inoculation because the cytokine profile induced by allergens may change with the time course of exposure[36] (see below). In that regard, comparing allergic rhinitic or atopic asthmatic subjects inoculated with RV in and out of the relevant pollen season would provide a better model to study interactions between RV infection and natural exposure to allergens.

Few reports on experimental colds in allergic subjects have assessed the timing of onset of cold symptoms. The usual incubation period (from virus inoculation to the onset of first cold symptoms) for experimental colds is 10 to 16 hours.[37] Doyle et al[38] compared nonallergic with allergic subjects out of pollen season infected with RV-39 and found that they had similar magnitude, frequency, and extent of symptoms. Although they noticed an earlier onset of sneezing (on day 1) and of eustachian tube obstruction (on day 2) in the allergic subjects, the overall changes during the colds were similar in the two groups and all other cold symptoms and physiologic measures (rhinomanometry, time of saccharin clearance, and spirometry) had similar magnitude and time of onset in both groups. The authors concluded that the severity of the colds was similar in allergic and nonallergic subjects. In our study all changes in symptoms and nasal secretions were delayed in subjects with allergic rhinitis who underwent allergen challenges before RV-16 inoculation.

Several mechanisms could explain the significant delay in the onset and the shortening in duration of colds in subjects primed with nasal allergen challenges. First,

the cytokine profile may change with the time course of allergen exposure. After intradermal injection of relevant allergens in atopic subjects, in situ hybridization studies have revealed that expression of IL-4 and IL-5 messenger RNAs (mRNA) increase within 6 hours and wane by 96 hours and that expression of IFN-γ and IL-2 mRNAs increase 48 to 96 hours after challenge.[36] Thus the repeated exposure to allergen in our subjects could have increased the production of IFN-γ and IL-2, which in turn could have increased the cytolytic activity of natural killer cells and cross-reactive cytotoxic T lymphocytes before RV-16 inoculation and led to attenuation of the common cold infection. Our inability to measure T_H1 and T_H2 cytokines in most of the nasal lavage samples precludes us from any direct analysis of this theory. Besides T_H1 cytokines, anti-inflammatory or down-regulatory cytokines such as IL-10 could have been secreted after repeated allergen challenges and inhibited the subsequent inflammatory response to the RV-16 infection.

A second mechanism that could explain the delay in onset and shortening of duration of colds caused by repeated allergen challenges before RV-16 inoculation is the local production of nitric oxide (NO). The nasal concentration of NO is increased in allergic rhinitic subjects[39,40] and further increases in the late phase response to allergen challenge.[41] Sanders et al[42] has demonstrated that NO inhibits rhinovirus-induced production of IL-6 and IL-8 and rhinovirus replication in epithelial cells. Therefore, the allergen challenges in our study could have inhibited RV-16–induced inflammation and infectivity indirectly by the induction of NO synthesis.

A third mechanism that could explain differences in cold severity between our 2 groups is the antiviral effects of eosinophil products. Eosinophil granular products such as eosinophil cationic protein and eosinophil-derived neurotoxin have ribonuclease activity and antiviral activity against RSV.[43] If these activities extend to rhinovirus, this could explain at least in part the inverse correlation we found in our study between percent eosinophils in nasal lavages immediately before RV-16 inoculation and severity of cold symptoms.

During acute common colds, we found increases in neutrophil percentage and IL-6 and IL-8 concentrations in nasal lavage fluid. These changes have been proposed as the mechanism by which cold viruses provoke symptoms.[10,44-47] Our finding of a delayed increase in nasal IL-6, IL-8, and neutrophil percentage paralleling the delayed appearance of cold symptoms in the group primed with nasal allergen challenges before RV-16 inoculation corroborates the current concept that the host response and not the virus causes cold symptoms.[48]

In summary, we found that priming the nasal mucosa with repeated allergen challenge before RV-16 inoculation does not worsen the severity of common cold symptoms but delays their onset and shortens their duration. These changes in cold symptoms were associated with parallel changes in the appearance and persistence of neutrophils, IL-6, and IL-8 in nasal secretions. These findings suggest that allergic rhinitis may not predispose people to more severe colds and indirectly suggest that environmental allergen exposure may not increase the likelihood of viral respiratory infection precipitating an asthma exacerbation.

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REFERENCES

1. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307:982-6.
2. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ 1995;310:1225-9.
3. Folkerts G, Busse WW, Nijkamp FP, Sorkness R, Gern JE. Virus-induced airway hyperresponsiveness and asthma. Am J Respir Crit Care Med 1998;157:1708-20.
4. Fleming HE, Little FF, Schnurr D, et al. Rhinovirus-16 colds in healthy and asthmatic subjects: similar changes in upper and lower airways. Am J Respir Crit Care Med 1999;160:100-8.
5. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. Am Rev Respir Dis 1976;113:131-9.
6. Lemanske RF Jr, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. J Clin Invest 1989;83:1-10.
7. Calhoun WJ, Swenson CA, Dick EC, Schwartz LB, Lemanske RF Jr, Busse WW. Experimental rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. Am Rev Respir Dis 1991;144:1267-73.
8. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995;151:879-86.
9. Trigg CJ, Nicholson KG, Wang JH, et al. Bronchial inflammation and the common cold: a comparison of atopic and non-atopic individuals. Clin Exp Allergy 1996;26:665-76.
10. Grunberg K, Timmers MC, Smits HH, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. Clin Exp Allergy 1997;27:36-45.
11. Cheung D, Dick EC, Timmers MC, de Klerk EP, Spaan WJ, Sterk PJ. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects in vivo. Am J Respir Crit Care Med 1995;152:1490-6.
12. Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. J Clin Invest 1994;94:2200-8.
13. Halperin SA, Eggleston PA, Beasley P, et al. Exacerbations of asthma in adults during experimental rhinovirus infection. Am Rev Respir Dis 1985;132:976-80.
14. Angelini B, Van Deusen MA, Doyle WJ, Seroky J, Cohen S, Skoner DP. Lower airway responses to rhinovirus-Hanks in healthy subjects with and without allergy. J Allergy Clin Immunol 1997;99:618-9.
15. Skoner DP, Doyle WJ, Seroky J, Fireman P. Lower airway responses to influenza A virus in healthy allergic and nonallergic subjects. Am J Respir Crit Care Med 1996;154:661-4.
16. Skoner DP, Doyle WJ, Seroky J, Van Deusen MA, Fireman P. Lower airway responses to rhinovirus 39 in healthy allergic and nonallergic subjects. Eur Respir J 1996;9:1402-6.
17. Grunberg K, Kuijpers EA, de Klerk EP, et al. Effects of experimental rhinovirus 16 infection on airway hyperresponsiveness to bradykinin in asthmatic subjects in vivo. Am J Respir Crit Care Med 1997;155:833-8.
18. Summers QA, Higgins PG, Barrow IG, Tyrrell DA, Holgate ST. Bronchial reactivity to histamine and bradykinin is unchanged after rhinovirus infection in normal subjects. Eur Respir J 1992;5:313-7.
19. Alper CM, Doyle WJ, Skoner DP, et al. Prechallenge antibodies: moderators of infection rate, signs, and symptoms in adults experimentally challenged with rhinovirus type 39. Laryngoscope 1996;106:1298-305.
20. Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. J Exp Med 1995;181:1229-33.

21. Coyle AJ, Bertrand C, Tsuyuki S, et al. IL-4 differentiates naive CD8+ T cells to a "Th2-like" phenotype: a link between viral infections and bronchial asthma. Ann N Y Acad Sci 1996;796:97-103.
22. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. Cell 1989;56:839-47.
23. Altman LC, Ayars GH, Baker C, Luchtel DL. Cytokines and eosinophil-derived cationic proteins upregulate intercellular adhesion molecule-1 on human nasal epithelial cells. J Allergy Clin Immunol 1993;92:527-36.
24. Bianco A, Spiteri MA. A biological model to explain the association between human rhinovirus respiratory infections and bronchial asthma. Monaldi Arch Chest Dis 1998;53:83-7.
25. Gern JE, Calhoun W, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. Am J Respir Crit Care Med 1997;155:1872-6.
26. Bardin PG, Fraenkel DJ, Sanderson G, et al. Amplified rhinovirus colds in atopic subjects. Clin Exp Allergy 1994;24:457-64.
27. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Immunohistochemical analysis of nasal biopsies during rhinovirus experimental colds. Am J Respir Crit Care Med 1994;150:1130-6.
28. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions, I: the common cold as a clinical entity. Arch Intern Med 1958;101:267-78.
29. Janson-Bjerklie S, Shnell S. Effect of peak flow information on patterns of self-care in adult asthma. Heart Lung 1988;17:543-9.
30. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine: an approach suitable for population studies. Am Rev Respir Dis 1987;136:1412-7.
31. Gwaltney JM Jr, Hendley O, Hayden FG, et al. Updated recommendations for safety-testing of viral inocula used in volunteer experiments on rhinovirus colds. Prog Med Virol 1992;39:256-63.
32. Knight V. The use of volunteers in medical virology. Prog Med Virol 1964;6:1-26.
33. Bush RK, Busse W, Flaherty D, Warshauer D, Dick EC, Reed CE. Effects of experimental rhinovirus 16 infection on airways and leukocyte function in normal subjects. J Allergy Clin Immunol 1978;61:80-7.
34. Robinson PJ, Hegele RG, Schellenberg RR. Allergic sensitization increases airway reactivity in guinea pigs with respiratory syncytial virus bronchiolitis. J Allergy Clin Immunol 1997;100:492-8.
35. Papadopoulos NG, Sanderson G, Hunter J, Johnston SL. Rhinoviruses replicate effectively at lower airway temperatures. J Med Virol 1999;58:100-4.
36. Tsicopoulos A, Hamid Q, Haczku A, et al. Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. J Allergy Clin Immunol 1994;94:764-72.
37. Harris JM, Gwaltney JM Jr. Incubation periods of experimental rhinovirus infection and illness. Clin Infect Dis 1996;23:1287-90.
38. Doyle WJ, Skoner DP, Fireman P, et al. Rhinovirus 39 infection in allergic and nonallergic subjects. J Allergy Clin Immunol 1992;89:968-78.
39. Martin U, Bryden K, Devoy M, Howarth P. Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis. J Allergy Clin Immunol 1996;97:768-72.
40. Garrelds IM, van Amsterdam JG, de Graaf-in't Veld C, Gerth van Wijk R, Zijlstra FJ. Nitric oxide metabolites in nasal lavage fluid of patients with house dust mite allergy. Thorax 1995;50:275-9.
41. Kharitonov SA, O'Connor BJ, Evans DJ, Barnes PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. Am J Respir Crit Care Med 1995;151:1894-9.
42. Sanders SP, Siekierski ES, Porter JD, Richards SM, Proud D. Nitric oxide inhibits rhinovirus-induced cytokine production and viral replication in a human respiratory epithelial cell line. J Virol 1998;72:934-42.
43. Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. Nucleic Acids Res 1998;26:3358-63.
44. Noah TL, Henderson FW, Wortman IA, et al. Nasal cytokine production in viral acute upper respiratory infection of childhood. J Infect Dis 1995;171:584-92.
45. Zhu Z, Tang W, Ray A, et al. Rhinovirus stimulation of interleukin-6 in vivo and in vitro: evidence for nuclear factor kappa B–dependent transcriptional activation. J Clin Invest 1996;97:421-30.
46. Teran LM, Johnston SL, Schroder JM, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. Am J Respir Crit Care Med 1997;155:1362-6.
47. Turner RB, Weingand KW, Yeh CH, Leedy DW. Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. Clin Infect Dis 1998;26:840-6.
48. Hendley JO. The host response, not the virus, causes the symptoms of the common cold. Clin Infect Dis 1998;26:847-8.