

Experimental rhinovirus challenges in adults with mild asthma: Response to infection in relation to IgE

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Background: Although most children and young adults with asthma are atopic, exacerbations of asthma are frequently associated with viral respiratory tract infections, especially those caused by rhinovirus (HRV).

Objective: Young atopic adults with mild asthma were evaluated before and during an experimental HRV infection to test the hypothesis that airway inflammation before virus inoculation may be a risk factor for an adverse response to HRV.

Methods: Experimental HRV infections were evaluated in 16 allergic volunteers with mild asthma and 9 nonatopic control patients (age, 18 to 30 years). Before virus inoculation, each participant was screened with tests for lung function, prick skin tests for sensitization to common aeroallergens, measurements of total serum IgE, and serum neutralizing antibody to rhinovirus-16 (the serotype used for inoculation). The response to infection was monitored for 21 days by using symptom diary cards, tests for lung function, and markers of airway inflammation in nasal washes, blood, and expired air.

Results: During the infection, asthmatic patients had cumulative upper and lower respiratory tract symptom scores that were significantly greater over the course of 21 days than scores from the control patients. At baseline, the asthmatic patients also had increased sensitivity to methacholine and significantly lower values for FEV₁ (percent predicted) than the control patients (geometric mean and intraquartile values: 87% [79% to 91%] for the asthmatic patients and 101% [90% to 104%] for the control patients, $P < .03$). Among the patients with mild asthma, 6 had levels of total serum IgE that were substantially elevated (range, 371 to 820 IU/mL) compared with 10 who had lower levels (range, 29 to 124 IU/mL). Those with high levels of IgE had significantly greater lower respiratory tract symptom scores during the initial 4 days of the infection than the low IgE group. They also had higher total

blood eosinophil counts at baseline, increased eosinophil cationic protein in their nasal washes (>200 ng/mL), and augmented levels of expired nitric oxide at baseline and during peak cold symptoms. In contrast, levels of soluble intracellular adhesion molecule-1 in nasal wash supernatants from the asthmatic patients with high IgE were diminished, both at baseline and during the infection.

Conclusions: The reduced lung function and increased markers of inflammation observed before virus inoculation in the asthmatic patients who had high levels of total serum IgE may be risk factors for an adverse response to infections with HRV. (J Allergy Clin Immunol 2003;111:1008-16.)

Key words: Asthma, rhinovirus, viral infections, IgE, allergic inflammation, eosinophils, eosinophil cationic protein, expired nitric oxide, intracellular adhesion molecule-1

Human rhinovirus (HRV), a viral pathogen strongly linked to the common cold, is also associated with exacerbations of asthma in school-age children and adults.^{1,2} In community and emergency room studies, at least 50% of children with asthma exacerbations have evidence for a recent HRV infection.^{1,3} Other studies indicate that most school-age children and young adults with asthma are atopic and have specific IgE antibody to common aeroallergens.³⁻⁵ Moreover, a significant correlation between levels of total serum IgE, the likelihood of having asthma, and airway hyperresponsiveness has been observed.^{6,7} However, the relative importance of and potential interactions between allergen and virus-induced inflammation in the airways remains unclear. Previous investigations have examined the host response to HRV in allergic and nonallergic individuals by using an experimental challenge model developed to study the pathogenesis of the common cold.⁸⁻¹² The purpose of these studies was to generate time-sequence information focused on molecular events that may be important determinants of an adverse response to HRV.

When adults with mild asthma are inoculated with HRV experimentally, they generally do not experience significant lower respiratory tract symptoms or marked changes in lung function.^{13,14} It can also be difficult to detect substantial differences in the generation of inflammatory cytokines stimulated by HRV in asthmatic patients compared with infected control patients.¹⁴ However, when HRV inoculation in atopic subjects is followed by an allergen challenge, enhanced bronchial hyperreactivity and an

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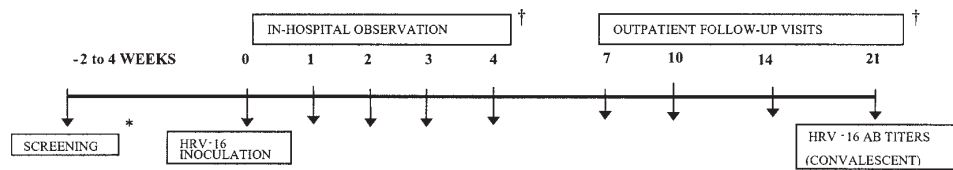


FIG 1. Experimental HRV study design. *Screening (2-4 weeks before HRV inoculation) included questionnaires, physical examination, spirometry, skin testing, nasal lavage, and blood for total IgE and serum antibody (AB) titers to HRV-16. †Upper and lower respiratory tract symptom scores and peak flows were recorded daily by patients on days 0 through 21. Sample collection on test days (marked with arrows) included nasal lavages, blood, spirometry, and expired air. Methacholine challenges were performed on days 0, 4, and 21.

Abbreviations used

BAL:	Bronchoalveolar lavage fluid
ECP:	Eosinophil cationic protein
eNO:	Expired nitric oxide
GCRC:	General Clinical Research Center
GM:	Geometric mean
HRV:	Human rhinovirus
RT-PCR:	Reverse transcriptase–polymerase chain reaction
sICAM-1:	Soluble intracellular adhesion molecule-1

augmented eosinophil response in bronchoalveolar lavage fluid (BAL) has been observed, and these abnormalities can be detected up to 1 month later.^{12,15} In contrast, when nasal allergen challenges have been given to allergic subjects before HRV inoculation, the onset of cold symptoms is surprisingly delayed and attenuated compared with the response in nonallergic control patients.¹⁶ These differences highlight the complexity of trying to understand the relation between allergic inflammation and the consequences of an HRV infection.

In the current study, young adults with mild asthma and nonatopic control patients were challenged experimentally with HRV (serotype 16). The atopic status of each patient was characterized by using prick skin tests to common aeroallergens and measurements of total serum IgE. The response to infection was monitored and compared in these patients with respect to respiratory tract symptoms, lung function, and markers of airway inflammation. The premise was that the asthmatic patients would have evidence for airway inflammation before HRV inoculation and that their risk for an adverse response to HRV during the infection would be increased.

METHODS

The participants included 16 young adults with mild asthma and 9 nonatopic patients without asthma. All patients were 18 to 30 years old. The asthmatic patients had symptoms consistent with a definition of mild asthma described in the Guidelines for the Diagnosis and Management of Asthma.¹⁷ More specifically, these patients had a history of physician-diagnosed asthma treated with inhaled bronchodilators (eg, albuterol) used intermittently. They did not require anti-inflammatory or other controller medications (ie, oral or inhaled steroids, cromolyn, nedocromil sodium, or leukotriene modifiers). None of the patients were ever intubated or treated in an intensive care unit for an exacerbation of asthma, and

none were hospitalized for asthma within 3 years before enrollment. The control patients had negative prick skin test reactions and no history of asthma, chronic lung disease, or allergy-related symptoms. None of the asthmatic patients or control patients had a history of an upper respiratory tract infection within 6 weeks of viral inoculation, and no patient was using nasal steroids. The study was approved by the Institutional Review Board at the University of Virginia, and all patients signed a consent form before participation.

Screening and virus inoculation

Each patient was evaluated in the clinic 2 to 4 weeks before virus inoculation (Fig 1). Questionnaires were administered to define their past and present medical history. Screening also included a physical examination, spirometry, and a nasal wash to confirm the absence of a recent HRV infection by reverse transcriptase–polymerase chain reaction (RT-PCR) and culture. A blood sample (5 mL) was obtained to measure total serum IgE and neutralizing antibody to HRV-16. Prick skin tests with common aeroallergens were performed by using extracts from Greer Pharmaceuticals (Lenoir, NC). The extracts used for testing included dust mite (*D farinae* and *D pteronyssinus*), cockroach, cat, dog, *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, mold mix, English plantain, eastern tree mix, oak, southern grass mix, weed mix, and ragweed allergens. Skin test responses 3 mm greater than a saline control were read as positive. Patients were infected with HRV-16 in the University of Virginia, General Clinic Research Center (GCRC). For virus challenge, each patient was inoculated while lying supine with 0.25 mL of HRV 16 in each nostril. The virus preparation was diluted in Hanks' balanced salt solution and was pretitered to contain 200 to 300 tissue culture infective doses [TCID₅₀] of HRV-16 per mL.

Study design

Samples obtained on the day of inoculation included nasal secretions, blood, and expired air (Fig 1). Peak flow tests and spirometry were performed, and bronchial reactivity to methacholine was determined by calculating the provocative concentration causing a 20% fall in FEV₁ (PC₂₀).¹⁸ Patients were monitored daily in the GCRC over the first 4 days. After discharge from the GCRC, they were seen in clinic on days 7, 10, 14, and 21. A physical examination focused on the respiratory tract was done on each study day. Nasal washes were collected with the use of a Yankauer suction catheter, as previously described.³ The nasal secretions were used for viral analyses (culture and RT-PCR) and for measurements of eosinophil cationic protein (ECP), soluble intracellular adhesion molecule-1 (sICAM-1), and albumin. Blood samples were analyzed for total blood eosinophil counts, serum ECP, and total serum IgE.³

Diary cards were scored daily for sore throat, sneezing, rhinorrhea, nasal congestion, wheeze, dyspnea, chest tightness, and cough by using a modification of the Jackson criteria.^{19,20} Symptoms characteristic of a common cold were judged to be present when volunteers had a symptom score of ≥5 or nasal discharge on 3 or more

days. Patients also recorded their peak flow measurements twice daily (before breakfast and dinner). Spirometry was done in the morning in the GCRC and at each follow-up visit, and methacholine challenges were done on days 0, 4, and 21. Expired air was collected for NO determinations before spirometry, as previously described.^{21,22} At the completion of the study (day 21), serum was tested for measuring neutralizing antibody to HRV-16.

Immunoassays and virology

Measurements of ECP and total serum IgE were done by using the Pharmacia CAP immunoassay (Uppsala, Sweden).³ Concentrations of sICAM-1 were done by using a 2-site sandwich immunoassay kit (R&D Systems, Minneapolis, Minn). Albumin was measured in nasal wash supernatants and sera by ELISA.²³ Viral cultures and RT-PCR for HRV-RNA in nasal washes were done as previously described.³

Statistical analyses

To attain an equal measurement variation across all subgroups, the measurements of nasal wash ECP, sICAM-1, expired nitric oxide (eNO), and total blood eosinophil counts were transformed to their respective natural logarithmic scale before statistical analysis. The value 1 was added to each of the observed values of ECP, eosinophil counts, and sICAM-1 before the logarithmic transformation to allow for samples that had undetectable levels of cells or mediators to be included in the analysis. These logarithmically transformed values were analyzed by mixed-effects analysis of variance (ANOVA) and mixed-effects analysis of covariance (ANCOVA),²⁴ statistical techniques that are specifically designed for modeling correlated data. The ANCOVA also allows for confounding factors such as the disparity between the subjects' baseline response to be taken into account in the parameter estimation process. With respect to model specification, the ANOVA and the ANCOVA models were hierarchical in structure, with the study group (asthmatic or control) treated as the between-subject factor and time treated as the within-subject factor. The subjects' baseline response on the day of virus inoculation functioned as the model covariate in the ANCOVA. The ANOVA and ANCOVA model parameters were estimated by restricted maximum likelihood, and an SP(POW) spatial variance-covariance structure was used to estimate the within-subject variance-covariance parameters, because this type of matrix allows the repeated measurements to be unequally spaced in time.²⁵ All of the ANOVA and the ANCOVA comparisons were conducted as a hypothesis test of the equality of the mean of the distribution of logarithmically transformed data, which is equivalent to a hypothesis test of the equality of the geometric mean (GM) of the distribution on the original scale. All of the ANOVA and the ANCOVA statistical analyses were carried out in SAS version 8.0 with the mixed-model software of PROC MIXED.²⁶

Total upper and lower respiratory tract scores were summarized for each individual by computing cumulative symptoms over study days 0 to 4 and over study days 0 to 21. The cumulative symptoms were analyzed as counts and modeled by generalized estimating equations; a statistical modeling technique suited for the analysis of nonnormally distributed correlated data.²⁷ Model parameter estimation was based on the maximum likelihood principle, and the variance-covariance matrix was estimated by the Huber and White estimator.²⁸ On the basis of the finding of our primary analyses, we performed post hoc analyses in which the original cohort of asthmatic patients were dichotomized according to the patient's baseline level of IgE. All outcome variables were reanalyzed with the study groups, consisting of 6 asthmatic patients with high IgE at baseline, 10 asthmatic patients with low IgE, and 9 control patients. The statistical methods used in the post hoc analyses were identical to those described for the primary analyses.

RESULTS

Response to HRV and atopic status of volunteers

On the basis of the Jackson criteria, all 16 asthmatic patients and the 9 control patients had upper respiratory tract symptoms characteristic of a common cold.^{19,20} Fifteen of the asthmatic patients and 6 control patients demonstrated seroconversion by day 21 (Table I). Over the course of 21 days, the asthmatic patients had cumulative upper respiratory tract symptom scores that were greater than the control patients. The mean (GM) and 95% CI for cumulative scores was 43.7 [28.8 to 66.3] for the asthmatic patients and 20.4 [12.8 to 32.7] for the control patients ($P < .03$). In addition, the development of peak symptoms in the asthmatic patients was in many cases delayed: for example, 8 of 9 control patients (89%) had peak symptoms during the first 4 days in the GCRC compared with 8 of 16 asthmatic patients (50%, $P = .05$). At baseline, the asthmatic patients also had increased sensitivity to methacholine, and their GM and intraquartile values for FEV₁ were significantly lower (87% [79% to 91%] predicted) than the control patients (101% [90% to 104%] predicted, $P < .03$). In addition, the asthmatic volunteers had higher total blood eosinophil counts, nasal ECP concentrations, and eNO levels both at baseline and during the infection, although the differences between asthmatic patients and control patients for these values were not statistically significant. In contrast, 6 of the asthmatic patients were noted to have substantially higher levels of total serum IgE at baseline (371 to 820 IU/mL) compared with the other 10 (29.2 to 124 IU/mL; Table I). The concentration of inflammatory markers (nasal ECP and eNO) at baseline and during the infection was amplified in the asthmatic patients with high IgE compared with the low IgE group and control patients. The results are presented below in a post hoc analysis. During the infection, total serum IgE levels initially increased by only 10% to 20% in both asthmatic groups and control patients and returned to baseline by day 7.

Upper and lower respiratory tract symptoms

At baseline and over the first 4 days in the GCRC, the cumulative upper respiratory tract symptom scores recorded by the asthmatic patients with high and low IgE levels and control patients were similar in severity. However, the delayed development of peak symptoms observed for the asthmatic patients was more pronounced in the low IgE group: 4 of 6 (67%) of the asthmatic patients with high levels of IgE had peak symptoms during the first 4 days compared with 4 of 10 (40%) of those with low IgE levels. During the 21 days of monitoring, asthmatic patients with high and those with low IgE had cumulative symptom scores that were increased and prolonged. The difference between scores for the high IgE group and control patients was significant ($P < .02$), whereas the difference between scores for the low IgE group and control patients was not ($P = .07$; Fig 2).

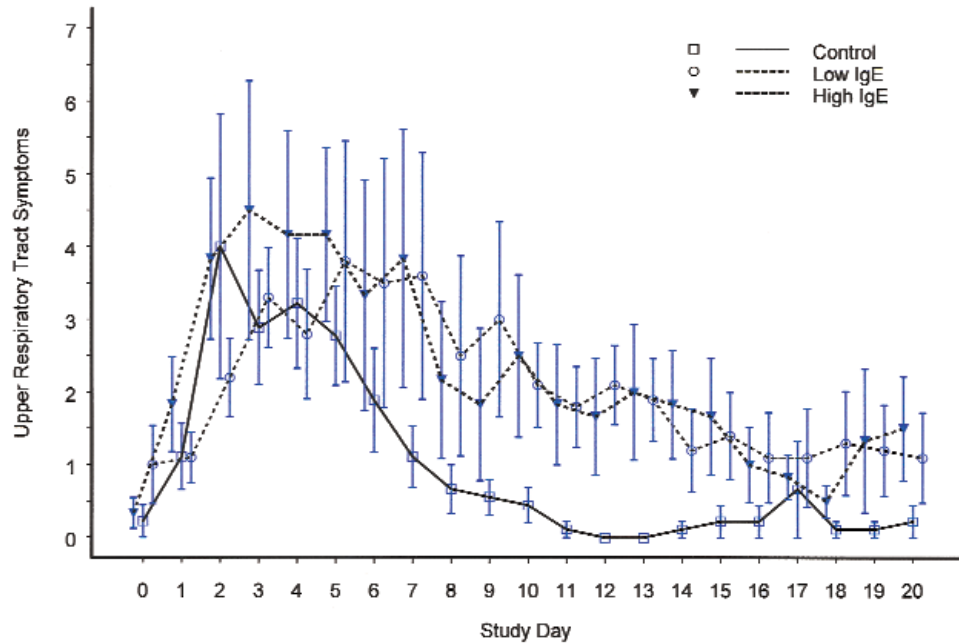


FIG 2. Upper respiratory tract symptom scores. Based on the Jackson criteria,^{19,20} the symptoms recorded daily by asthmatic and controls included sore throat, sneezing, rhinorrhea, and nasal congestion. Vertical lines on this and other figures represent standard error bars.

TABLE I. Patient characteristics

Age	Sex	RV antibody titer†		RV detection		FEV ₁	% Pred.	IgE IU/mL	Skin test results‡
		Pre	Post	RT-PCR	Culture				
Asthmatic subjects*									
26	F	1:2	≥ 1:64	+	+	2.80 L	80	383	DM, cr, cat, dog, rgw
25	M	1:2	≥ 1:32	+	+	3.99 L	90	371	DM, tr, oak, gr, we, rgw
22	F	1:2	≥ 1:32	+	+	2.46 L	78	820	DM, cat, tr, gr, ep, we, rgw
18	F	≤ 1:2	1:32	+	+	2.24 L	72	414	DM, cr, tr, oak, gr, we
30	F	1:4	≥ 1:32	+	+	2.67 L	84	568	DM, cat, alt, gr, we, rgw
22	M	≤ 1:2	≤ 1:2	+	+	3.23 L	79	434	DM, cr, cat, tr, oak, gr
23	F	1:4	≥ 1:64	+	+	3.13 L	82	53.3	Cat, gr
24	M	1:4	1:16	+	+	5.20 L	123	29.2	DM, cr, cat, alt, tr, oak, gr, rgw
29	F	1:2	≥ 1:64	+	+	2.45 L	78	95.9	DM, cat, tr, oak, gr, we, rgw
23	M	1:2	1:32	+	+	4.15 L	91	124	DM, cat, tr, oak, gr
20	F	≤ 1:2	1:4	+	+	3.58 L	90	76.8	DM, cr, tr, oak, ep, rgw
18	M	≤ 1:2	1:4	+	+	4.64 L	111	58.8	DM
29	M	≤ 1:2	≥ 1:32	+	+	5.49 L	117	56.1	Cr, alt, clad, pen, mm, tr, oak, gr, ep, we, rgw
24	M	≤ 1:2	≥ 1:32	+	+	3.85 L	83	108	DM, cat, alt, tr, gr, rgw
26	F	≤ 1:2	1:16	+	+	2.89 L	91	58.9	DM
25	F	≤ 1:2	≥ 1:32	+	+	2.71 L	85	82	DM
Control subjects									
22	F	< 1:2	1:16	+	+	3.81 L	114	6.56	Negative
24	M	1:2	< 1:2	+	+	4.96 L	101	11.4	Negative
20	M	1:4	≥ 1:64	+	+	4.36 L	100	23.1	Negative
30	M	≤ 1:2	1:2	+	+	3.94 L	93	19.8	Negative
24	M	≤ 1:2	≥ 1:32	+	+	5.71 L	123	27.8	Negative
18	F	≤ 1:2	1:32	+	+	2.76 L	94	11.4	Negative
27	F	≤ 1:2	1:2	+	+	3.14 L	76	6.6	Negative
21	F	≤ 1:2	≥ 1:32	+	+	3.39 L	104	23.6	Negative
20	M	1:2	1:4	+	+	3.67 L	87	37.8	Negative

DM, Dust mite; gr, grass; alt, ; we, weed; rgw, ragweed; cr, cockroach; tr, tree; clad, Cladosporium; asp, Aspergillus; pen, Penicillium; mm, mold mix; ep, English plantain.

*The first 6 asthmatic subjects listed had significantly higher levels of total serum IgE than the other 10 asthmatic subjects ($P < .001$).

†Titers of neutralizing antibody to HRV-16 prior to virus inoculation on day 0 (Pre) and at the last follow-up visit on day 21 (Post).

During the infection, all but 3 of the asthmatic patients reported mild lower respiratory tract symptoms of cough, wheeze, shortness of breath, or chest discomfort. Only one subject (an asthmatic in the low IgE group) had a mild cough at baseline. By day 4, cumulative lower respiratory tract symptom scores for the asthmatic patients with high IgE were greater (mean [CI]: 4.2 [1.4 to 12.5]) than for the asthmatic patients with low IgE (1.0 [0.4 to 2.5], $P = .05$) and control patients (0.4 [0.2 to 1.2], $P = .003$) (low IgE group versus control patients, $P = .2$). For asthmatic patients with high IgE and those with low IgE, these scores were significantly increased ($P < .001$) compared with control patients by day 21 (high IgE = 13.5 [6.2 to 29.3]; low IgE = 8.5 [3.2 to 22.3]; control patients = 0.7 [0.3 to 1.5]). None of the asthmatic patients had symptoms requiring intervention with anti-inflammatory medications (ie, inhaled or oral steroids).

Assessments of lung function and expired NO

The FEV₁ values among subjects with high levels of IgE were reduced at baseline before HRV inoculation (GM = 78.8% predicted) compared with the patients with low IgE levels (93.8% predicted, $P < .03$) and control patients (101.8% predicted, $P < .002$). The baseline FEV₁ values in the asthmatic patients with low levels of IgE and control patients were not significantly different ($P = .2$). The high IgE group also had lower peak flow test results before inoculation, but no significant changes from baseline were observed for peak flow tests or FEV₁ values within any group during the infection (data not shown). The asthmatic volunteers showed significantly greater reactivity to methacholine than the control patients before and during the HRV infection (Fig 3, A). The GM values at baseline for asthmatic patients with high IgE and those with low IgE and control patients were 1.2, 1.6, and 13.3 mg/mL, respectively ($P < .02$ for asthmatic patients with high IgE and those with low IgE versus control patients; asthmatic patients with high IgE and those with low IgE, $P = .7$). The provocative dose for methacholine among the asthmatic patients remained lower but did not change significantly during the infection. The GM of eNO levels detected at baseline were 15.3, 10.6, and 8.5 ppb for the asthmatic patients with high IgE and those with low IgE and control patients ($P < .04$, asthmatic patients with high IgE versus control patients) (Fig 3, B). During peak symptoms, the maximal peak (ppb) for patients within each group were 25.1 for the asthmatic patients with high IgE, 16.1 for the asthmatic patients with low IgE, and 12.7 for control patients ($P < .05$ for the patients with high IgE compared with both the patients with low IgE and control patients; patients with low IgE compared with control patients, $P = .4$).

ECP and blood eosinophil counts

Mean GM values for ECP in nasal washes were 189, 62, and 18 ng/mL at baseline in the high and low IgE asthmatic patients and control patients, respectively ($P < .003$ for the high IgE group compared with control patients; P

= .06 for the low IgE group compared with control patients) (Fig 4, A). Maximal values for nasal ECP during the infection for the high and low IgE asthmatic patients and control patients were 769, 228, and 172 ng/mL, respectively ($P < .05$, high IgE group compared with control patients). When the magnitude of change in nasal ECP concentrations during the infection was compared among the three groups of volunteers (ie, maximal ECP concentrations minus baseline levels before virus inoculation), no significant differences were observed between groups. However, when the concentrations of nasal ECP were related to a high level that was previously reported in asthmatic patients during acute wheezing exacerbations (ie, ≥ 200 ng/mL) significant differences were clear.³ Over the first 4 days of infection after HRV inoculation, levels of ECP > 200 ng/mL were detected in 62% (15/24) of nasal washes from the high IgE asthmatic patients compared with 32% (13/40) of washes from the low IgE asthmatic patients ($P < .05$) and 11% (4/36) of washes from the control patients ($P < .001$).

Total blood eosinophil counts were also elevated at baseline in the high IgE patients (GM = 226 cells/mm³) compared with the low IgE group (81 cells/mm³) and control patients (95 cells/mm³). The difference was significant between the high and low IgE groups ($P < .04$). During the infection, the GM of maximal eosinophil values for patients in the high and low IgE groups and control patients were 353, 184, and 197 cells/mm³, respectively (not statistically different between the 3 groups). Serum ECP increased significantly from GM = 11.4 ng/mL at baseline to 27.2 ng/mL on day 3 in the high IgE asthmatic patients ($P < .05$), whereas the increase from baseline for the other 2 groups was not significant.

Nasal wash sICAM-1

In contrast to nasal ECP, the concentrations of nasal sICAM-1 were lower at baseline and during peak symptoms in the asthmatic patients with high levels of IgE (Fig 4, B). The GM of maximal sICAM-1 values during the infection among the high and low IgE asthmatic patients and control patients were 8.7, 16.0, and 20.8 ng/mL, respectively. To test whether the lower nasal sICAM-1 concentrations in the high IgE asthmatic patients might be influenced by vascular leakage associated with inflammation, albumin was measured in the nasal wash supernatants. The albumin levels increased 6-fold within each of the study groups but were not significantly different in the asthmatic patients (high or low IgE) compared with the control patients.

DISCUSSION

Among the asthmatic patients who were infected with HRV in this study, two distinct groups were observed with high and low levels of total serum IgE. The analysis of data based on levels of total IgE was influenced by population surveys that have shown that most school-age children and young adults with asthma are atopic and that higher levels of total serum IgE are significantly

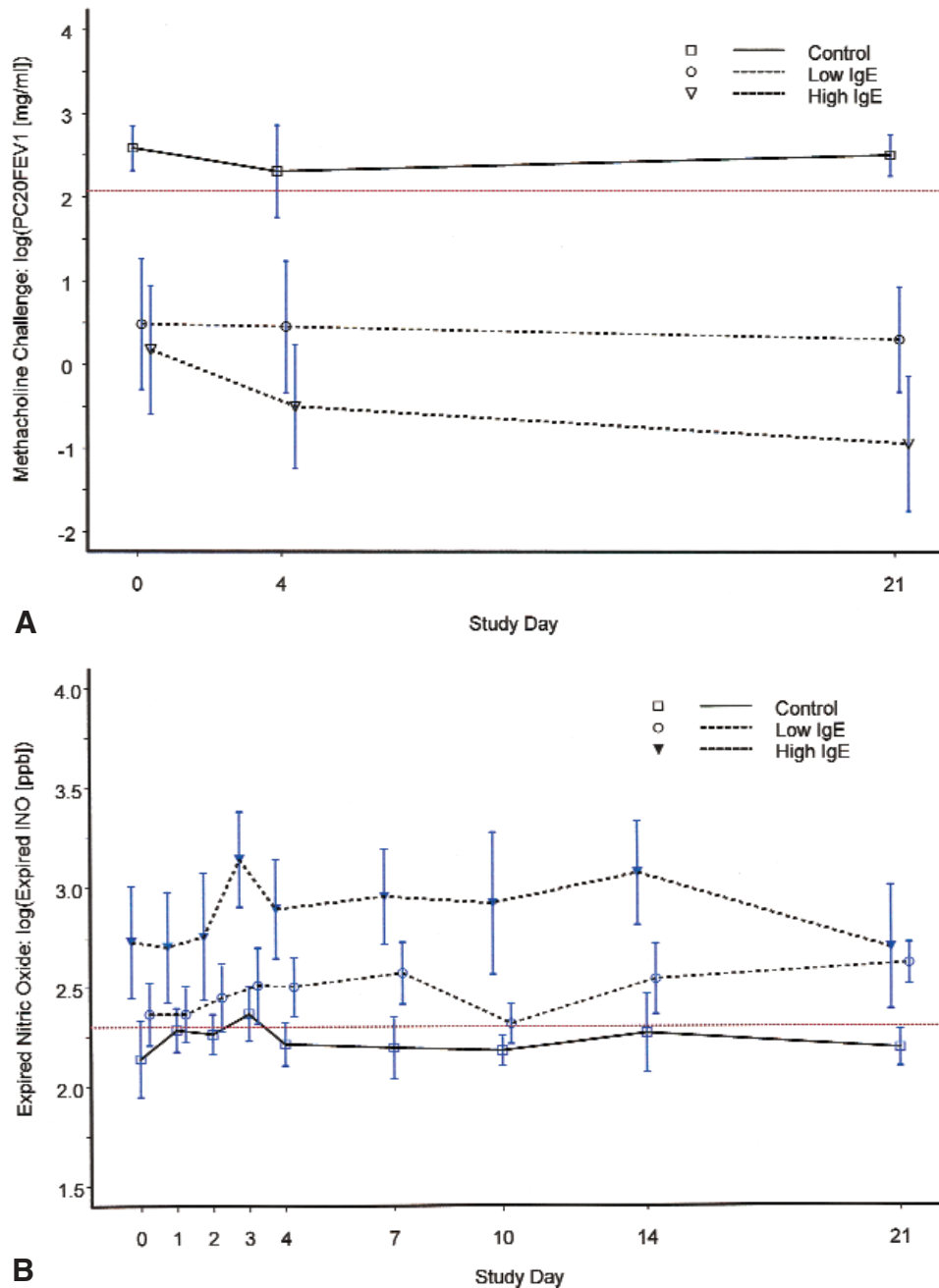


FIG 3. Assessments reflecting changes in the lower airway during the HRV infection. **A**, Methacholine challenges were done on day 0 (before HRV inoculation), day 4 (the day of discharge from the clinical research center), and day 21. Concentrations of methacholine (mg/mL) causing a 20% fall in FEV₁ are plotted on a natural logarithmic scale. A 20% fall in FEV₁ at a methacholine concentration of <8 mg/mL (horizontal hatched line) is characteristic of bronchial hyperresponsiveness in asthma.¹⁸ **B**, Expired NO (eNO) levels (in ppb) are also plotted on a natural logarithmic scale. Levels of eNO above 10 ppb (horizontal hatched line) are detected more frequently in children and adults with asthma.^{21,22}

associated with the likelihood of having asthma and bronchial hyperreactivity.³⁻⁷ In keeping with this, the asthmatic patients with high IgE levels in our study had increased sensitivity to methacholine and significantly lower values for FEV₁ before HRV inoculation and during the infection. These volunteers also had positive prick skin test reactions to a minimum of 5 aeroallergens

reflecting both indoor and seasonal allergen exposures. However, 6 of the asthmatic patients with low levels of IgE also had 5 or more positive prick skin test reactions, but their lung function tests and inflammatory markers at baseline and during the infection were very similar to the asthmatic patients with low IgE and only one or two positive skin tests (data not shown).

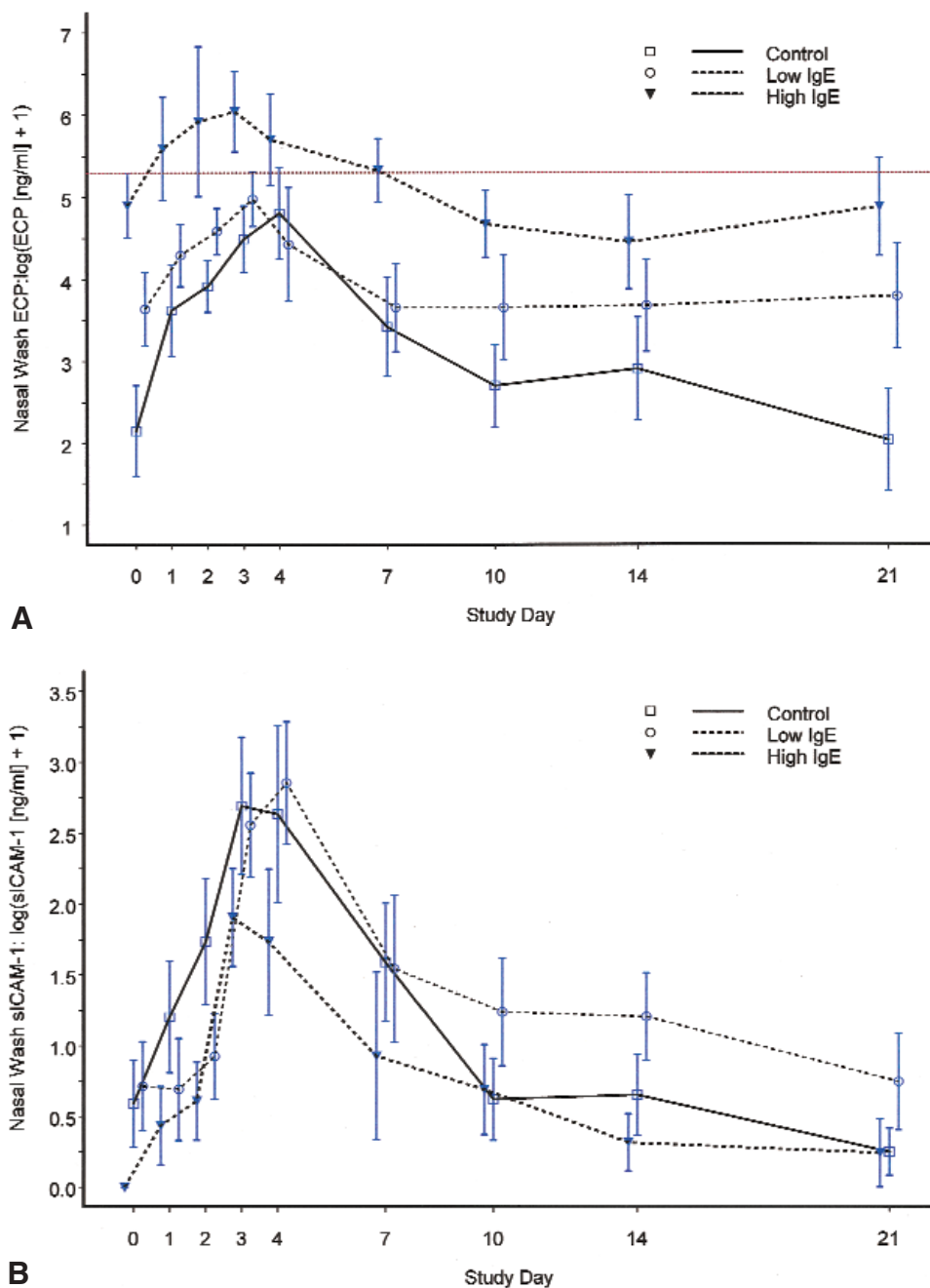


FIG 4. Assessments in nasal washes (**A**, ECP; **B**, sICAM-1). ECP concentrations greater than 200 ng/mL (*horizontal hatched line*) have been observed more frequently in asthmatic individuals with acute attacks of wheezing than in controls.³ The values for both ECP and sICAM-1 were transformed to the natural logarithmic scale before analysis.

The magnitude of upper respiratory tract symptoms during the initial phase of infection was not significantly different in the asthmatic volunteers and control patients. Although amplified colds in allergic individuals inoculated with HRV have been described previously,⁹ the asthmatic patients in our study had cold symptoms that were only more prominent and persistent than the control patients during the latter stages of the infection. The asth-

matic patients, especially those with low levels of IgE, also had cold symptoms that peaked later in the infection, similar to the response observed in a previous study of allergic subjects who were inoculated with HRV after nasal allergen challenge.¹⁶ Although the augmented upper respiratory tract symptoms reported by the asthmatic patients over 21 days may have been aggravated by returning home to allergen exposures after being dis-

charged from the GCRC, the asthmatic patients, particularly those with high IgE levels, reported lower respiratory symptoms that began in the GCRC during the initial 4 days of the infection. In previous studies, the development of lower respiratory tract symptoms has been observed, but treatment intervention has been uncommon.^{11,29} Treatment interventions were not required in our study, nor were significant reductions in lung function noted. This may be attributed to the fact that experimental HRV challenges have only been administered to individuals with mild asthma. There is little information to know whether adults with mild asthma would be more likely to have lower respiratory tract symptoms or significant changes in lung function with different strains of HRV or during natural HRV colds.

The asthmatic patients with high levels of IgE also had increased levels of nasal ECP before virus inoculation. They also had levels of ECP that were markedly elevated (>200 ng/mL) compared with the low IgE group or control patients during peak respiratory tract symptoms. In addition, the high IgE asthmatic patients had significantly increased levels of eNO, a marker of lower airway inflammation in asthmatic patients,^{21,22} both at baseline and during the infection. By comparison, the levels of nasal ECP, blood eosinophil counts, and eNO did not differ significantly between the asthmatic patients with low IgE levels and control patients before or during the infection. These results provide evidence that airway inflammation was present in the high IgE group before virus inoculation even though their nasal symptom scores and respiratory tract findings on physical examination were similar to the other patients at baseline. The persistence of increased markers of inflammation in the high IgE asthmatic patients is also consistent with findings in BAL and bronchial mucosal biopsies obtained from atopic patients who were infected with HRV experimentally and who still had evidence for an augmented eosinophil response in samples obtained 1 month after the onset of infection.^{15,30}

In contrast to nasal ECP, the levels of nasal sICAM-1 were reduced in the high IgE asthmatic patients both at baseline and during the infection compared with levels in the other volunteers. Previously, IFN- γ and IL-1 have been shown to augment the cell surface expression of ICAM-1.³¹ However, in the milieu of augmented inflammation, which may be induced by Th₂ mechanisms in the high IgE asthmatic patients, the capacity to generate increased amounts of IFN- γ as well as ICAM-1 may be impaired. This possibility is consistent with reports that IFN- γ levels during HRV infection may be decreased in atopic individuals, with implications that an effective cytopathic (Th₁ and Tc₁) lymphocyte response required to promote viral clearance may be hampered.^{32,33} The rationale for monitoring levels of nasal sICAM-1 in this investigation was based on its role as the receptor for most strains of HRV, including HRV-16, on airway epithelial cells.³⁴ In addition, ICAM-1 is an important adhesion molecule involved in the trafficking of leukocytes, predominantly neutrophils, which have been observed in large numbers in nasal secretions during

peak cold symptoms with HRV infections.³⁵ Activated eosinophils also express the ligand for ICAM-1 (LFA-1) on their cell surface, and other studies indicate that neutrophils also might contribute to the production of ECP.³⁶ Thus, the observation that ECP is increased in nasal washes from the control patients in this study during peak symptoms may not be surprising.

In summary, high levels of total serum IgE in the asthmatic patients challenged experimentally with HRV were associated with reduced lung function and increased markers of airway inflammation (eg, nasal ECP and eNO) before virus inoculation. During the initial 4 days of infection, the asthmatic patients had lower respiratory tract symptoms that were augmented in the high IgE group. In addition, the asthmatic patients with high IgE had levels of nasal ECP and eNO that were significantly elevated during their peak respiratory tract symptoms compared with both the asthmatic patients with low IgE levels and the control patients. After peak symptoms, these inflammatory markers also remained elevated in the high IgE group during the course of the infection. In previous studies, we observed that actively wheezing children and adults in our emergency room frequently had positive test results for HRV along with significantly elevated levels of total serum IgE.^{3,22,37} These observations, together with results from the current study, support the hypothesis that asthmatic patients who are highly atopic might be more likely to have chronic or persistent airway inflammation and therefore have a greater risk for asthma exacerbations induced by HRV.

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