

Exercise-induced asthma is not associated with mast cell activation or airway inflammation

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Exercise-induced asthma (EIA) may affect up to 90% of patients with asthma. Hyperpnea associated with exercise leads to increased airway water and heat loss, which contributes to the development of EIA. Measurement of circulating mediators has suggested that mast cells may participate in the development of EIA via release of histamine and neutrophil chemotactic factor. To evaluate further the contribution of pulmonary mast cell-mediator release in the pathogenesis of EIA and to determine whether EIA is associated with enhancement of airway inflammation, we studied 11 subjects with mild stable asthma (FEV_1 , $93\% \pm 3\%$ predicted; mean $\pm$ SEM) with significant EIA (after exercise fall in FEV_1 , $41\% \pm 5\%$). Bronchoalveolar lavage (BAL) was performed immediately (less than 1 hour) after exercise challenge (EC) and repeated 24 hours later (exercise studies). On another occasion, paired BALs were done 24 hours apart (control studies). A minimum of 2 weeks separated the exercise and control pairs. No changes were observed in BAL cell counts, differentials, or reactive oxygen species metabolism after EC. Neither BAL histamine nor BAL tryptase levels increased, either shortly (less than 1 hour) or 24 hours after EC. We conclude that EC in subjects with asthma is not associated with cellular influx to airspace and that mechanisms other than histamine release by pulmonary mast cells may be responsible for EIA. (J ALLERGY CLIN IMMUNOL 1992;89:60-8.)

Key words: Exercise, asthma, histamine, bronchoscopy, mast cell, bronchoprovocation

EIA is symptomatic bronchospasm that develops shortly after strenuous exercise. Most patients with asthma have EIA when the exercise is of sufficient duration and intensity.¹ Despite its prevalence and significance, the precise mechanisms responsible for EIA remain unknown. Because hyperpnea-induced heat and water loss leads to evaporative and convective airway cooling that extends as far as the eighth to the tenth generation of the bronchial tree, airway cooling

Abbreviations used

EIA:	Exercise-induced asthma
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
EC:	Exercise challenge
HBSS:	Hanks' balanced salt solution
AM:	Alveolar macrophages
LPR:	Late-phase reaction
HR:	Histamine release
MDI:	Metered-dose inhaler
PMA:	Phorbol myristate acetate

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has been proposed to be a stimulus causing EIA.^{1,2} Furthermore, water loss may act directly by increasing osmolarity of epithelial lining fluid to cause bronchoconstriction.³ However, it is not clearly understood how the physical stimulus (airway cooling or drying) leads to bronchoconstriction. On the basis of increased levels of circulating histamine and neutrophil chemotactic factor, (a putative mast cell mediator)⁴ after EC in atopic subjects with asthma, Lee et al.⁵ sug-

gested that physical stimulus leads to mast cell degranulation and mediator release, which in turn caused bronchoconstriction. However, other investigators have not observed a rise in plasma histamine during isocapnic hyperventilation in subjects with asthma.⁶ Recently, Broide et al.⁷ demonstrated the lack of measurable changes in levels of mast cell mediators in BAL after EC that raised additional questions regarding the role of pulmonary mast cells in the pathogenesis of EIA. Pulmonary mast cell activation with the release of neutrophil chemotactic factor can lead to recruitment of neutrophils to the airway, thus enhancing airway inflammation; however, this has not been evaluated in earlier studies.^{4, 5} BAL has emerged as a powerful tool for understanding inflammatory lung diseases. BAL in subjects with asthma is safe^{8, 9} and informative since it provides direct access to airway cells and mediators. Bronchial biopsy may provide a better assessment of mucosal inflammation than BAL, but BAL is sensitive and less invasive. In addition, BAL allows the evaluation of mediators that are potentially important in asthma that a biopsy specimen does not. With BAL technique in atopic subjects with asthma, allergen challenge has been demonstrated to cause airway inflammation and mediator release.¹⁰⁻¹³ Atopic subjects with asthma demonstrated a significant rise in BAL eosinophils and total cell recovery after relevant antigen challenge.^{10, 14} In addition to the increase in airspace cell numbers after antigen challenge, we have demonstrated an increase in their chemiluminescence, suggesting enhanced inflammatory potential of these cells.¹³ Other investigators have demonstrated that enhanced chemiluminescence of AMs correlates with the clinical severity of asthma.¹⁵ Local antigen challenge causes a rise in the levels of histamine and tryptase in BAL immediately after challenge, suggesting that mast cells are activated by airway antigen challenge.^{12, 16} Thus, we hypothesized that changes in BAL histamine and tryptase levels would be observed after EC if mast cell degranulation was important in the pathogenesis of EIA. The purpose of this study was to evaluate the role of pulmonary mast cell mediator release in EIA and the possible contribution of EC to airway inflammation in subjects with mild, stable asthma.

MATERIAL AND METHODS

Subject selection and characterization

Volunteers with asthma, between 18 and 45 years of age, were recruited for this study, which was approved by the University of Wisconsin Committee for Human Subjects. Informed consent was obtained from each subject before each bronchoscopy and BAL. Each subject had a preliminary history, physical examination, spirometry, and a

screening EC before enrollment. Only subjects with baseline $FEV_1 > 70\%$ predicted and a fall in $FEV_1 > 20\%$ after EC were included in the study. Subjects were excluded if they were smokers, receiving cromolyn or steroid therapy, or if they had a contraindication to EC. We performed bronchoscopy and BAL in 11 subjects with mild, stable asthma, immediately and 24 hours after EC. Ten subjects were known to have a positive skin test to common aeroallergens. Detailed subjects' characteristics are presented in Table 1. Eight of these subjects also had paired BALs performed 24 hours apart without a prior EC. The three subjects who were included in the exercise study but not in the control study were similar to the remaining eight subjects in age, baseline pulmonary function, severity of FEV_1 fall after EC, and medication use. The results of the control lavages have been published in detail.¹⁷

Study design

Each subject was studied by BAL immediately (less than 1 hour) and 24 hours after EC. The EC immediately before the first BAL was guided by each subject's performance during his screening EC. Spirometry was obtained before and at 5, 10, and 15 minutes after EC. All subjects reached the maximum fall in FEV_1 at 5 or 10 minutes after EC. Albuterol (two puffs by MDI) was administered at that time to relieve symptoms of bronchospasm and as a premedication for bronchoscopy. All subjects experienced immediate symptomatic relief of bronchospasm, and, by the time of bronchoscopy, were free from cough or wheezing. After the completion of BAL, subjects were kept in the General Clinical Research Center for observation. Vital signs and spirometry were checked hourly for a minimum of 2 hours. To control for any effects that airway instrumentation and BAL may have had, we performed paired control BALs 24 hours apart without EC. Four subjects had the exercise pair first, followed by the control pair. In each pair, however, the first BAL was done from the right middle lobe and the second BAL from the lingula. The studies were separated by at least 2 weeks to obviate any influence of the first pair of BAL studies on the second pair.

EC and pulmonary functions

EC was performed on a treadmill with a ramp protocol. Subjects breathed unconditioned room air (temperature, 22° to 24° C; relative humidity, 15% to 55%). A single lead ECG was used for continuous monitoring. After a 1- to 2-minute "warm-up" (1 mph and 0° elevation), the speed and/or elevation were increased every 2 minutes until 80% of maximum predicted heart rate ($0.8 \times [220 - \text{age in years}]$) was reached. Exercise was maintained at that level for 4 to 6 minutes for a total exercise duration of 10 to 12 minutes.¹⁸ Exercise was terminated earlier if the subject complained of leg fatigue, marked dyspnea, or chest pain. A spirometer (model PS 600, Puritan-Bennett, Madison, Wis.) was used to obtain spirometry before and after EC. An FVC maneuver was performed, and the best of two or three trials was accepted according to the American Thoracic Society guidelines.¹⁹ Spirometry was obtained before exercise and then at 5, 10, 15, 20, 30, and 60 minutes after

TABLE I. Subjects' characteristics

Subject No.	Age/sex (yr)	FEV ₁ /L			% Fall*	Medications
		Predicted	Baseline	After EC		
1	40/M†	4.08	3.32	1.67	50	T,B
2	24/M	4.31	3.63	1.39	62	B
3	21/M†	4.78	4.63	3.63	22	B
4	33/F	3.19	3.37	1.30	61	T,B
5	37/F	2.65	2.40	1.74	28	T,B
6	28/M†	4.18	3.85	2.09	46	B
7	19/M	4.45	4.23	3.22	24	B
8	42/M	4.10	3.12	1.18	62	B
9	23/M	4.90	4.63	3.09	33	T,B
10	22/F	3.38	3.39	2.65	22	B
11	38/M	4.51	4.74	2.61	45	T,B
Mean ± SEM	30 ± 3	4.05 ± 0.21	3.76 ± 0.22	2.23 ± 0.26‡	41 ± 5	

T, Theophylline; B, β_2 -agonist MDI.

*FEV₁ percent fall after EC.

†Subjects who participated in the exercise study only.

‡ $p < 0.0001$ before versus after EC.

screening EC. The response to EC was determined by maximum fall in FEV₁ after EC compared to baseline value:

$$[(\text{FEV}_1 \text{ (baseline)} - \text{FEV}_1 \text{ (after EC)}) / (\text{FEV}_1 \text{ (baseline)})] \times 100 = \text{percent fall in FEV}_1$$

After spirometry returned to baseline, subjects were allowed to leave the exercise room and were instructed to return at 2, 4, and 6 hours after EC for a repeat spirometry if they had any symptoms suggestive of bronchospasm. EC was performed before BAL in a fashion similar to that of the screening challenge. Spirometry was obtained every 5 minutes after EC until a maximum fall occurred; subsequently, preparation for bronchoscopy proceeded immediately.

Bronchoscopy and BAL

Subjects were instructed to stop their oral theophylline (24 hours) and β -agonist inhaler (12 hours) before BAL. All bronchoscopies were done by one of the authors (N. N. J.) between 7:30 AM and 8:30 AM, as previously described.^{17, 20}

Premedications included intramuscular atropine sulfate (0.6 mg), midazolam (1.25 mg), and inhaled albuterol (two puffs). The fiberoptic bronchoscope (Olympus Corp. of America, New Hyde Park, N.Y.) was wedged into a subsegment of the right middle lobe on the first study and on the lingula on the second study. Lavage was performed by instilling and immediately withdrawing four aliquots (60 ml each) of sterile, nonpyrogenic, 0.9% NaCl at 37° C.

BALF processing

The lavage return was pooled without filtering through gauze, and BAL cells were recovered by centrifugation. Supernatant fluid was pooled and frozen at -70° C. The cell pellet was washed once in phosphate-buffered saline and resuspended in HBSS. Cells were counted with a hemo-

cytometer and were brought to a final dilution of 2×10^6 cells per milliliter for use in cytofluor preparation and cell-functional assays. Cytofluor slides were air-dried, fixed in methanol, and stained (Diff-Quik; Scientific Products, Chicago, Ill.). Differential cell counts were made by identifying ≥ 300 cells as lymphocytes, neutrophils, eosinophils, or macrophages. Lymphocyte surface markers were identified with commercially available monoclonal antibodies (OK series; Ortho Diagnostics, Raritan, N.J.) and flow cytometric analysis (FACStar; Becton Dickinson, Mountain View, Calif.).

Total protein, histamine, and tryptase assays

BALF protein was measured by an assay according to the method of Lowry et al.,²¹ modified for microtiter equipment with a sensitivity of 20 $\mu\text{g/ml}$.²² BALF histamine was measured by a single isotope radioenzymatic assay with purified rat kidney histamine *N*-methyltransferase,²³ with a linear coefficient of correlation of 0.99 and a sensitivity of 30 pg/ml. Values below the level of sensitivity were analyzed statistically as 30 pg/ml. BALF tryptase was measured in whole BALF by a sandwich ELISA method based on a modification²⁴ of a previously described procedure.^{12, 25} The murine monoclonal antibody, G5, for capture, and the biotinylated murine antibody, G4, for detection, were used.²⁴ The assay lower limit of detection is 0.05 ng of tryptase per 40 μl sample. Samples were concentrated up to 20-fold by ultrafiltration with a Centricon 10 chamber (Amicon Corp., Danvers, Mass.).

Airspace cells superoxide assay

Superoxide anion production by BAL cells was quantified by a spectrophotometric method based on the superoxide dismutase-inhibitable reduction of ferricytochrome *c*, adapted for 96-well microtiter assay.²⁶ Each well contained

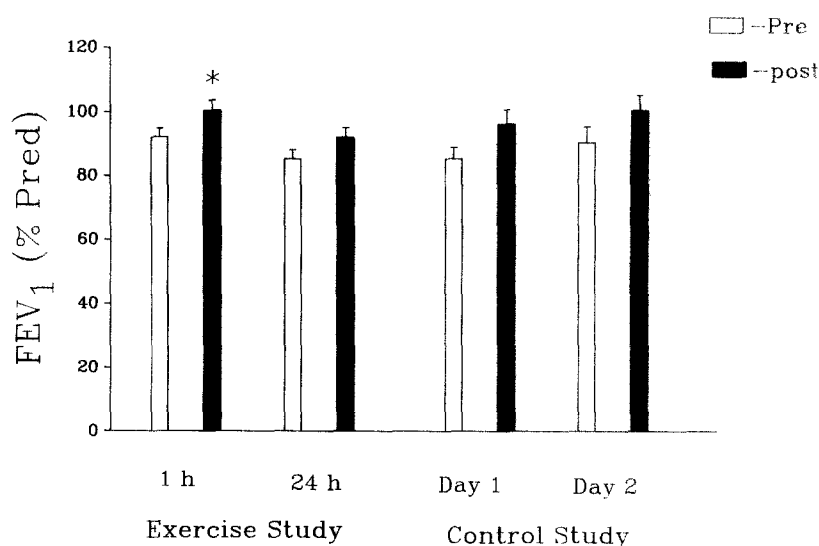


FIG. 1. Spirometry. FEV₁ (percent predicted) before and after bronchoscopy on each of the study days. Note that there was a tendency toward higher FEV₁ percent predicted after bronchoscopy; *Pre*, before BAL; *post*, after BAL. Data are presented as mean $\pm$ SEM; * $p < 0.05$ after versus before.

10^5 cells, 0.1% gelatin, 1.2 mg/ml cytochrome *c*, and HBSS. Paired wells were established with 25 μ g/ml of superoxide dismutase, and each cell preparation was assayed under three conditions: (1) no activator, (2) opsonized zymosan (1 mg/ml), and (3) 20 μ g/ml of PMA. The mean of duplicate basal and stimulated superoxide anion produced at 60 minutes was used for calculations.

AM chemiluminescence assay

Chemiluminescence responses of adherence-purified AMs were determined at 37° C in a Picolite (Packard Instrument Co., Downer's Grove, Ill.) model 6500 luminometer interfaced to a laboratory computer.²⁰ Briefly, 2×10^5 cells were allowed to adhere to a glass vial in the presence of HBSS and autologous serum. Nonadherent cells were aspirated, and the adherent cells refed with HBSS and gelatin. Luminol-enhanced (10 μ mol/L) chemiluminescence determinations were made on unstimulated cells and after addition of PMA (20 μ g/ml) or opsonized zymosan (0.5 mg/ml). Samples were run in duplicate, and light output was measured, starting immediately after adding the activator and every 5 minutes for 60 minutes. Data were expressed in thousands of counts per minute. Peak chemiluminescence occurred generally in about 10 minutes. The mean of basal- and peak-stimulated chemiluminescence measurements was used for calculations.

Data and statistical analysis

Data were first analyzed in a microcomputer spreadsheet (SuperCalc 4; Computer Associates, San Jose, Calif.). Subsequent analysis was performed with a microcomputer statistics package (Abstat 5; Anderson-Bell, Parker, Colo.). All data are expressed as mean $\pm$ SEM. The results from the 4 study days were compared with analysis of variance,

and additional comparisons were done by paired and independent Student's *t* test unless it is otherwise noted. A *p* value of <0.05 was considered significant.²⁷

RESULTS

No complications were encountered during EC or BAL. Minor complaints included transient cough, fatigue, and sore throat.

EC and pulmonary functions

Baseline FEV₁ (percent predicted) values were similar on all 4 study days. Before bronchoscopy and BAL, EC was performed to an average of 86% of maximum predicted heart rate with a fall in FEV₁ of $41\% \pm 5\%$ from baseline (range, 22% to 62%; Table I) after this EC. Screening EC was associated with a similar fall in FEV₁ ($36\% \pm 5\%$; $p = 0.67$, Student's paired *t* test versus before bronchoscopy EC). The EC was associated with cough, dyspnea, and wheezing, especially in subjects whose fall in FEV₁ exceeded 50%. However, in these subjects, the symptoms quickly resolved after the administration of an albuterol MDI (two puffs). On the day of EC, the FEV₁ after BAL was significantly ($p < 0.05$) higher than the prebronchoscopy value. Thus, despite the magnitude of the EC, subjects tolerated the BAL well with no worsening of airflow limitation after BAL (Fig. 1). FEV₁/FVC after bronchoscopy was higher than before bronchoscopy value on each of the 4 study days ($p < 0.03$ before versus after bronchoscopy for each comparison). The improvement in spirometry after BAL is most likely related to bronchodilator ef-

TABLE II. Basic BAL analysis*

	Exercise study (n = 11)		Control study (n = 8)		ANOVA
	1 hr	24 hr	Day 1	Day 2	
Volume (ml)	178 ± 4	176 ± 5	166 ± 9	175 ± 7	0.59
TP	65 ± 5	72 ± 5	76 ± 9	73 ± 6	0.62
Total cells§	13.8 ± 2.5	16.0 ± 2.9	11.7 ± 2.2	14.9 ± 2.5	0.71
Macrophages†	92.0 ± 0.9	93.8 ± 1.2	91.5 ± 2.6	91.7 ± 1.5	0.67
Lymphocytes†	6.5 ± 1.0	3.5 ± 0.9	7.5 ± 2.4	7.1 ± 1.4	0.16
Neutrophils†	0.7 ± 0.3	0.7 ± 0.2	0.6 ± 0.15	0.7 ± 0.33	0.97
Eosinophils†	0.8 ± 0.4	1.0 ± 0.6	0.4 ± 0.20	0.5 ± 0.23	0.74
T4 Lymphocytes	37.7 ± 4.6¶	45.0 ± 3.7¶	40.8 ± 6.9‡	42.4 ± 6.5#	0.76
T8 Lymphocytes	35.5 ± 5.8¶	29.4 ± 3.1¶	34.2 ± 6.9#	30.7 ± 9.0#	0.87

TP, Total protein in micrograms in milliliters.

Excluding lymphocyte proportions and numbers, there were no significant differences among these data from the 4 different days.

*Data are expressed as mean ± SEM.

†Percent of total cells.

§In millions.

|| $p < 0.05$; exercise 1 hour versus exercise 24 hours.

Number of samples in which lymphocyte markers were done:

¶n = 9 samples.

‡n = 7 samples.

#n = 6 samples.

fect of bronchoscopy premedications (albuterol and atropine).

BALF

Volume return and total protein concentration were similar for all 4 study days (Table II). BAL total cell recovery and percentages of eosinophils and neutrophils were similar in the lavage samples from the 4 study days (Table II). No influx of eosinophil or neutrophil cells was observed as a result of EC, either immediately or 24 hours later. BAL lymphocytes were lower in percentage and absolute numbers in the lavage done at 24 hours after EC compared to that in BAL done immediately after EC ($3.5\% \pm 2.7\%$ versus $6.5\% \pm 3.1\%$; $p < 0.05$); however, no significant differences in lymphocyte subsets (OKT4 and OKT8) were observed among the four BAL studies (Table II). Thus, EC was not associated with evidence of cellular influx or increased vascular leak. Bronchoscopy and BAL induced no significant changes in the BALF obtained 24 hours later, as previously reported.¹⁷

Airspace cell reactive oxygen species metabolism (Table III)

There were no significant differences in the basal or stimulated release of superoxide anion by airspace cells among the four study days. Superoxide release was enhanced by opsonized zymosan and PMA on each of the study days compared to basal levels

($p < 0.001$, zymosan-stimulated versus basal; $p < 0.0005$, PMA-stimulated versus basal superoxide release on each of the 4 study days). Peak basal and stimulated chemiluminescence of adherence-purified AMs were not significantly different among the 4 study days. In general, chemiluminescence increased with PMA or zymosan stimulation compared to unstimulated levels; however, the increase was not uniformly significant (Table III).

BAL histamine and tryptase

BAL histamine concentrations in subjects with asthma were slightly higher than in normal subjects (113 ± 22 pg/ml; control subjects with asthma, $N = 8$, versus 41 ± 6 pg/ml; healthy nonatopic subjects, $N = 8$). However, eight of the 38 BAL samples from this study were at, or below, the sensitivity level of our assay (30 pg/ml). In one subject, histamine level was 1050 pg/ml on the first control day; this value was excluded from the final analysis since it was higher than the mean by >10 SD. (Analysis done with that value included revealed essentially similar results.) Histamine levels in BAL obtained shortly (less than 1 hour) after EC (119 ± 31 pg/ml) were not significantly different from levels obtained on control days 1 or 2 (113 ± 22 and 56 ± 17 pg/ml, respectively) or levels on the second day of BAL after EC (83 ± 24 pg/ml) (Fig. 2). Histamine levels were lower in the second day of BAL of the control pair. BAL tryptase levels were similar on both control days

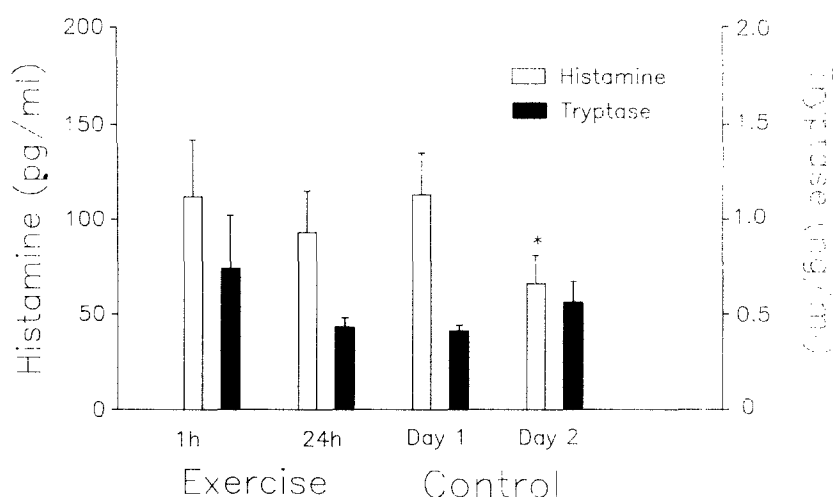


FIG. 2. BAL histamine and tryptase levels. Data are presented as mean $\pm$ SEM. In control pair, BAL histamine was lower on day 2 versus day 1 ($p < 0.05$). No other significant differences were observed.

TABLE III. Reactive oxygen species*

	Exercise study (n = 11)		Control study (n = 8)	
	1 hr	24 hr	Day 1	Day 2
Superoxide (nmol/500,000 cells/hr)				
Basal	2.3 $\pm$ 0.5†	3.4 $\pm$ 0.8	3.5 $\pm$ 0.8	3.7 $\pm$ 0.7
PMA	9.4 $\pm$ 1.3†§	10.5 $\pm$ 1.6§	9.9 $\pm$ 1.2§	9.6 $\pm$ 1.4§
ZYM	8.6 $\pm$ 0.7†§	10.1 $\pm$ 1.2§	9.7 $\pm$ 1.2§	9.9 $\pm$ 1.4
Chemiluminescence (Kcpm)				
Basal	191 $\pm$ 101	123 $\pm$ 27	113 $\pm$ 24‡	100 $\pm$ 25
PMA	695 $\pm$ 242¶	418 $\pm$ 137¶	1371 $\pm$ 684‡	306 $\pm$ 60#
ZYM	4339 $\pm$ 2354	2466 $\pm$ 1539	3929 $\pm$ 1780‡	1119 $\pm$ 252#

Zym, Zymosan; Kcpm, Thousand counts per minute.

No significant differences exist among the 4 study days.

*Data are expressed as mean $\pm$ SEM.

Number of BAL samples in which the assays were done are 11 in the exercise study and eight in the control study, with two exceptions:

†n = 10 samples.

‡n = 7 samples.

§p < 0.0005 stimulated versus basal.

||p < 0.001 stimulated versus basal.

¶p < 0.05 stimulated versus basal.

#p < 0.01 stimulated versus basal.

(0.41 $\pm$ 0.03 versus 0.56 $\pm$ 0.11 ng/ml). After EC, no significant increase in BAL tryptase was observed either immediately (0.74 $\pm$ 0.28 ng/ml) or 24 hours later (0.43 $\pm$ 0.05 ng/ml).

DISCUSSION

In this study, 11 subjects with asthma underwent bronchoscopy and BAL immediately and 24 hours after EC. No rise in BAL histamine or tryptase was observed at either time after EC. Furthermore, EC did

not lead to an increase in BAL total cell numbers, granulocyte percentage, total protein concentration, or reactive oxygen species metabolism of airspace cells. From these observations, we suggest that EIA is not associated with mast cell mediator release or enhancement of airway inflammation.

Although the association of severe asthma with airway inflammation is not a new observation, the association of mild asthma and airway hyperresponsiveness with airway inflammation has been recog-

nized only in recent years.²⁸ BAL is a powerful tool in the investigation of pulmonary inflammation. In atopic subjects with asthma, antigen challenge has been demonstrated to cause marked increase in BAL cell numbers and activity starting 4 hours after the challenge that persist for 96 hours or longer.¹⁰⁻¹² Allergen challenge is frequently associated with a late response that can be prolonged, severe, or recurrent.²⁹ In contrast, EC leads to a short-lived, self-limited, single response, and is infrequently associated with late-phase response or prolonged worsening of obstruction.^{29, 30} We have demonstrated that antigen challenge in a similar group of subjects with asthma is associated with increased BAL eosinophils, histamine, and total protein, as well as enhanced reactive oxygen species metabolism.¹³ In the current study, EC was not associated with a change in these indices despite a significant bronchoconstriction that was more severe than what we observed in the antigen-challenge study.¹³ The marked difference in biologic response to the two stimuli suggest that the mechanisms by which antigen challenge and EC produce bronchoconstriction may be different. Alternatively, the lack of airway inflammation following EC may be related to the lack of LPR after EC, since LPR did not occur in any of the 11 subjects in this study after EC. Whether or not these subjects would respond with LPR to antigen was not addressed by this study.

Histamine released from mast cells and basophils is an important mediator of immediate hypersensitivity. Plasma histamine levels were found to correlate with the severity of asthmatic exacerbations, suggesting that histamine may play a role in the pathogenesis of asthma.³¹ Lee et al.⁴ described a rise in plasma histamine during EC and postulated that it was released from lung mast cells that are stimulated by exercise. However, Deal et al.⁶ could not find consistent changes in plasma histamines after isocapnic hyperventilation. Furthermore, Howarth et al.³² found that subjects with asthma had a higher plasma histamine than normal subjects and that exercise in subjects with asthma led to increased basophils and total blood histamine. They concluded that the previously reported rise in plasma histamine associated with EC may have been related to HR from peripheral blood basophils from sample handling rather than intrapulmonary mast cell degranulation. From these and other studies,³³ the link between EC and pulmonary mast cell mediator release could not be drawn conclusively. Even if mast cell mediators are elevated in peripheral blood during exercise, it is still difficult to prove that pulmonary mast cells were the source for these mediators. Thus, recent studies have focused on the re-

lease of mediators into the airway, the measurement of which became possible with the use of BAL.

BAL histamine has been found to be elevated in subjects with asthma and to correlate with the degree of airway responsiveness³⁴ and severity of airway obstruction.³⁵ Endobronchial allergen challenge in atopic subjects with asthma was associated with a significant increase in BAL histamine concentration that was not observed after nonspecific challenge with cold saline or antigen challenge in normal subjects.¹⁶ Furthermore, the increase in BAL histamine levels after endobronchial antigen challenge correlated closely with the rise in levels of BAL tryptase, a specific marker for mast cell degranulation, suggesting that allergen challenge activated pulmonary mast cells in atopic subjects with asthma.¹² A similar rise in BAL histamine and tryptase might be expected if pulmonary mast cell degranulation is involved in the pathogenesis of EIA.

In a recent study of seven atopic subjects with asthma studied by BAL before and after EC (leading to FEV₁ drop of >15%), no significant differences were found between the mean BAL histamine, tryptase, prostaglandin D₂, and leukotriene C₄, before and after exercise.⁷ This study suggested that EIA, unlike allergen-induced asthma, may occur by mast cell-independent mechanisms. In our study, no changes were observed in the levels of BAL histamine or tryptase after EC. Thus, our data corroborate data of Broide et al.⁷ demonstrating no evidence of mast cell activation in association with EIA.

One explanation for the lack of BAL histamine rise after challenge is that HR may have occurred in our subjects but was not detected in BALF, since it took about 30 minutes from the time of exercise to obtain BAL samples. It is possible that any putatively released histamine may have been absorbed or metabolized by that time. It was not possible to obtain airway samples closer to EC in our study design, but the timing would have allowed the detection of tryptase, which has a longer half-life. Alternatively, β -agonists that are known to inhibit mast cell mediator release may have prevented HR from pulmonary mast cells; however, since albuterol was administered after successful EC, when postulated HR would have already occurred, this is unlikely to be a valid explanation. Furthermore, we have detected rises in BAL histamine after antigen challenge with similar techniques, including premedication with inhaled β -agonist.¹³ Another possible explanation is that mast cell degranulation does not occur during EC and that other mechanisms are more important in provoking EIA. This suggestion is supported by the lack of a rise in BAL

histamine or tryptase, the lack of cellular influx 24 hours after EC in our study, and the absence of change in these and other mast cell mediators (prostaglandin D₂ and leukotriene C₄) in BAL after EC in a previous study.⁷

In summary, EIA was not associated with mast cell mediator release or increased inflammatory cells in the airspace. These data suggest that mediator release by pulmonary mast cells may not be important in the pathogenesis of EIA and that EIA and allergen-driven asthma may be pathogenically different.

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Elevation of IgE in HIV-infected subjects: A marker of poor prognosis

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*The IgE synthesis is tightly controlled by a complex network of T and B cells. Because human immunodeficiency virus (HIV) disease associates T cell activation and depletion, polyclonal B cell activation, atopic symptoms, drug hypersensitivity, and autoimmune activity, we have evaluated IgE, as well as IgA, IgG, and IgM, in 315 HIV-seropositive individuals with or without acquired immunodeficiency syndrome (AIDS) and compared the results to those of 100 HIV-seronegative subjects. IgE levels were higher in HIV-infected subjects as a whole, compared to levels in seronegative control subjects ($p < 0.05$). This difference was particularly marked between patients with AIDS and control subjects ($p < 0.005$). A strong relationship appeared between IgE and the immune status as assessed by CD4 cell counts ($p < 0.001$ between IgE values in patients with $CD4 < 300$ or $> 300/\mu L$). In addition, we assessed the predictive value of IgE elevation over disease progression: in subjects with a CD4 count $< 300/\mu L$, the survival analysis disclosed a 24-month occurrence rate of AIDS of 83% in individuals with $IgE > 150$ KIU/L versus 44% in individuals with $IgE < 150$ ($p = 0.016$). In subjects with an AIDS-related complex, $IgE > 150$ indicated a 100% rate of AIDS versus 9% in individuals with $IgE < 150$ ($p = 0.003$). Thus, IgE levels appear to be a very discriminative marker between patients in late stages of HIV infection. IgA, IgG, and IgM also significantly increased during HIV infection; the slope of their increase was, however, significantly less steep than that of IgE, suggesting different mechanisms of hyperproduction. Hypotheses regarding these mechanisms are discussed. (*J ALLERGY CLIN IMMUNOL* 1992;89:68-75.)*

Key words: IgE, HIV

Hypergammaglobulinemia affecting the IgG, IgA, IgM, and IgD has been repeatedly reported as a common feature of HIV disease.¹⁻⁷ In contrast, the rela-

tionship between IgE and HIV has elicited rise to fewer observations.^{1, 8-12}

We had previously studied this relationship⁹ because the IgE synthesis is tightly controlled by a complex network of T and B cells and lymphokines¹³⁻¹⁵ and is known to markedly increase in atopy, primary immune deficiencies,¹⁶ viral or parasitic infections, and AGVHD, as well as in autoimmune disorders.¹⁷ We therefore believed that IgE regulation should be severely disturbed in HIV disease, since this disorder associates polyclonal B cell activation, major T cell dysfunction, multiple superinfections, atopic manifestations, drug hypersensitivity, and features of au-

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