

Elevated plasma eotaxin levels in patients with acute asthma

Craig M. Lilly, MD,^a Prescott G. Woodruff, MD,^{b,d} Carlos A. Camargo, Jr, MD,^{b,d}
Hidetoshi Nakamura, MD,^a Jeffrey M. Drazen, MD,^a Eric S. Nadel, MD,^c and John P.
Hanrahan, MD^{b,e} *Boston, Mass*

Background: The eosinophil chemotactic and activating effects of eotaxin and the known association of eosinophils with asthma suggest that eotaxin expression is increased during asthma exacerbations.

Objective: We sought to determine whether plasma eotaxin levels are elevated in patients presenting for emergency treatment of acute asthma and to correlate eotaxin levels with disease activity and responses to treatment.

Methods: A case-control study of plasma eotaxin levels was performed in the 46 patients who presented for emergency asthma treatment and 133 age-, sex-, and ethnicity-matched subjects with stable asthma.

Results: Plasma eotaxin levels were significantly higher in 46 patients with acute asthma symptoms and airflow obstruction (520 pg/mL [250, 1100 pg/mL]; geometric mean [-1 SD, $+1$ SD]) than in 133 subjects with stable asthma (350 pg/mL [190, 620 pg/mL]; $P = .0008$). Among the patients with emergency asthma flares, those who responded to asthma treatment with an increase in peak expiratory flow rate by an amount equal to at least 20% of their predicted normal value had lower eotaxin levels than those who did not (410 pg/mL [210, 800 pg/mL] and 660 pg/mL [300, 1480 pg/mL], respectively; $P = .04$).

Conclusion: These findings imply that eotaxin either is mechanistically involved in acute asthma or serves as a biomarker for activity of the CCR3 receptor ligand system, which is functionally linked to asthma. (*J Allergy Clin Immunol* 1999;104:786-90.)

Key words: Chemokines, emergency medicine, lung, pulmonary diseases

Asthma is characterized by recurrent dyspnea, cough, wheeze, and airflow obstruction mediated by airway inflammation¹⁻³ in which a variety of cell types, includ-

Abbreviation used

PEFR: Peak expiratory flow rate

ing eosinophils,⁴ mast cells,⁵ and T lymphocytes,⁶ contribute to a complex pathologic process that causes airway remodeling and ultimately leads to compromised lung function. In recent years, interest in the role of eotaxin in asthma pathogenesis has grown.

Eotaxin is a chemotactic cytokine or chemokine of the CC class that recruits eosinophils by activating the CCR3 chemokine receptor. Activation of leukocyte chemokine receptors is part of the process of leukocyte extravasation that involves selectins, integrins, and chemokines. Eotaxin is one member of a growing family of ligands for the CCR3 receptor that is present on cell types associated with asthma, including eosinophils,⁷ basophils,⁸ and T lymphocytes.⁹ Eotaxin is produced by airway epithelial cells after stimulation by cytokines,¹⁰ and its airway expression is induced by allergen exposure in sensitive asthmatic subjects.¹¹ In asthmatic airways eotaxin has been localized immunohistochemically and by *in situ* hybridization analysis to eosinophils, macrophages, and epithelial and endothelial cells.^{12,13} After allergen challenge, eotaxin is expressed on the epithelial surface of the airways in sites at which eosinophils accumulate.¹¹ Airway eotaxin levels are directly related to the sensitivity of asthmatic airways to contractile stimuli and to the presence of eosinophils in the airways of subjects with mild asthma.¹³ Eotaxin levels have been directly associated with an asthma diagnosis and found to be inversely related to lung function, as measured by FEV₁.¹⁴ The established chemotactic¹⁵ and activating¹⁶ effects of eotaxin on eosinophils and the association of eosinophils with asthma activity and severity suggest that eotaxin is an important mediator in acute asthma. Studies linking plasma eotaxin to an asthma diagnosis and impaired lung function¹⁴ led us to the following 2 hypotheses: (1) plasma eotaxin levels are increased in asthma exacerbations and (2) a direct exposure-response relationship exists between the clinical severity of an asthma exacerbation and the level of plasma eotaxin. To address these 2 hypotheses, we compared the plasma eotaxin levels of 46 adult patients with asthma seeking emergency care for an asthma exacerbation with those of a matched sample of 133 patients with stable asthma.

From ^athe Combined Program in Pulmonary and Critical Care Medicine, ^bthe Channing Laboratory, Department of Medicine, and ^cthe Department of Emergency Medicine, Brigham and Women's Hospital, Boston; ^dthe Department of Emergency Medicine, Massachusetts General Hospital, Boston; and ^ethe Department of Emergency Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston.

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Reprint requests: Craig M. Lilly, MD, Respiratory Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

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METHODS

Study population

Participants in this study included 46 adults with physician-diagnosed asthma who presented to 3 emergency departments in Boston hospitals for treatment of an asthma exacerbation, as well as 133 adult asthmatic control subjects without symptoms of an asthma exacerbation. Subjects in the asthma flare patient group were recruited over a period of 2 weeks from the emergency departments of Massachusetts General Hospital, Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center.

Each of 43 patients with an exacerbation of asthma was matched with 3 control subjects with stable asthma of the same sex, ethnicity, and age (± 3 years). For the remaining 3 patients, 3 suitable, matched control subjects satisfying these criteria were not available from the pool of stable asthmatic control subjects, and 1 or 2 control subjects were used instead. Data from asthmatic patients for whom no matching control subjects could be identified were not included in this analysis. The control subjects with asthma were recruited at the same time as the patients through newspaper advertisements. The eotaxin levels for some of the control subjects have been reported.¹⁴ Respondents included in the control group for this investigation had documented reversibility of airflow reduction characterized as an improvement in FEV₁ of at least 15% after the administration of an inhaled short-acting β -agonist. In addition, these control subjects had been free of symptoms of a respiratory infection and of any asthma exacerbation for at least the previous 4 weeks and had not used either inhaled or systemic corticosteroids, nedocromil, or cromolyn for at least 1 month before evaluation. All control subjects had a normal chest radiograph within 1 year before enrollment. Asthma symptoms in the control group were managed with an inhaled short-acting β -agonist (albuterol), and nasal symptoms were managed by administration of loratadine. None of the control subjects were taking long-acting β -agonists, ipratropium bromide, astemizole, terfenadine, cetirizine, hydroxyzine, oral β -agonists, aspirin, nonsteroidal anti-inflammatory drugs, or nasal steroids. All subjects in this study provided written informed consent using documents approved by the respective institutional review boards.

Subjects from 18 to 54 years of age with acute asthma flares were enrolled on presentation to the emergency department in one of the 3 participating hospitals. Concomitant with the initial clinical evaluation and the initiation of emergency therapy with oxygen and nebulized β -agonists with or without systemic corticosteroids, a plasma specimen was obtained from each patient, and a standardized questionnaire was administered by a trained investigator. We used the Multicenter Asthma Research Collaboration Study¹⁷ questionnaire specifically developed for gathering information from patients presenting to emergency departments with asthma flares. Specifically, the Multicenter Asthma Research Collaboration Study instrument collects demographic information on patients with acute asthma and detailed data on the patients' asthma history, including age at symptom onset, frequency of symptoms and flares, medications, hospitalizations, and asthma triggers. It also documents the duration and severity of the current exacerbation, peak expiratory flow rate (PEFR) data before and after treatment, and emergency department treatment and disposition.

For all participants, initial emergency department therapy for the acute asthma exacerbation included nebulized short-acting β -agonists and oxygen. This treatment and subsequent therapy were ordered by the examining emergency medicine physicians, who also made decisions regarding the hospital admission or discharge of the patient from the emergency department.

Study measurements

Pulmonary function. PEFR was measured at presentation for emergency care and after β -agonist inhalation but before departure from the emergency department. The measurements were obtained with hand-held peak-flow meters used routinely for the assessment of asthma in the emergency departments of the 3 participating hospitals, with the peak-flow maneuver supervised by either a trained respiratory therapist or a participating investigator. All instruments met the standards of the American Thoracic Society and were used in accordance with the instructions of the manufacturers. All measurements for each subject were made on the same instrument. The predicted peak flow was calculated for each subject by the method of Roca et al,¹⁸ and subjects whose PEFR increased by an amount that was at least 20% of their predicted normal value during their stay in the emergency department were identified.

Plasma eotaxin assay. Plasma was collected in EDTA-containing tubes at the time of presentation for emergency treatment of asthma in the patient group and at the time of enrollment in the control group. Plasma was separated from cells within 2 hours and stored at -20°C until assay. Eotaxin levels were determined in duplicate as previously described; the ELISA could detect 10 pg/mL with a coefficient of variation of 10%.^{10,14} In addition to the standard curve, 3 standard plasma samples with varying known concentrations of eotaxin were added to each plate, and interplate variation was reduced by allowing calibration (correction) of the standard curve for differences between individual plates. This assay system accurately detects the addition of exogenous eotaxin to human plasma and therefore probably detects both bound and free eotaxin.

Statistical analysis

All analyses were performed by using Stata 5.0 (Stata Corp, College Station, Tex) or SAS 6.12 software (SAS Institute, Cary, NC). Characteristics of patients were compared by contingency-table methods or *t* tests for continuous variables. Serum eotaxin values were converted to base-10 logarithms to satisfy normality assumptions. Eotaxin levels are therefore presented as geometric means followed by the mean minus 1 SD and plus 1 SD. Trends in the eotaxin level according to symptom-treatment group were compared by a test for trend across ordered treatment groups.¹⁹ All *P* values presented are 2-tailed.

RESULTS

Demographic information

Features of the patients with acute asthma exacerbations and of stable asthmatic control subjects who were enrolled in this investigation are summarized in Table I. Of participating asthmatic subjects, nearly three fourths were women, 26% were African American, and 13% were Hispanic. Fifty-five patients with acute asthma were enrolled in the study; plasma for eotaxin assay was available for 52, and matching with at least one control asthmatic subject was possible for 46. At presentation for emergency asthma care, 1 subject was taking oral steroids, 7 subjects were taking both oral and inhaled corticosteroids, 16 subjects were taking inhaled steroids, and 22 subjects were taking neither form of steroids.

Plasma eotaxin levels

Main findings. Plasma eotaxin levels were significantly higher in subjects with acute asthma (520 pg/mL [250,

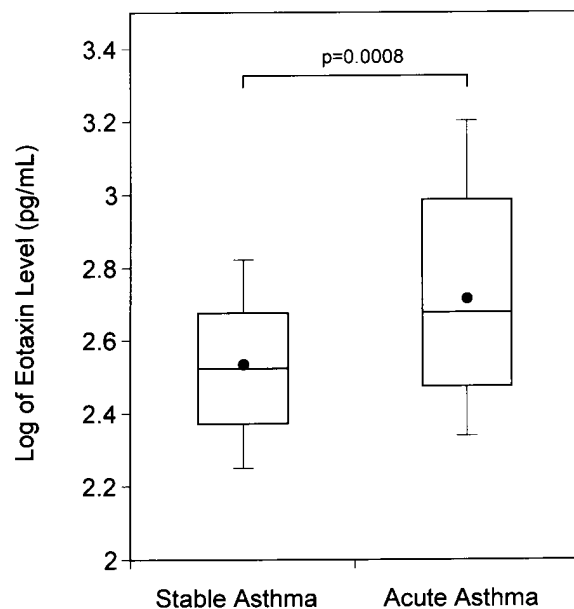


FIG 1. Median plasma eotaxin levels, interquartile range, and upper and lower adjacent values after log transformation for patients with acute asthma ($n = 46$) and control subjects with stable asthma ($n = 133$).

TABLE I. Demographic characteristics of subjects with acute asthma (patients) and stable asthma (control subjects) in a study of plasma eotaxin levels and asthma

	Acute asthma ($n = 46$)	Stable asthma ($n = 133$)	<i>P</i> value
Age, y (mean $\pm$ SD)	34 $\pm$ 11	33 $\pm$ 11	.93*
Sex, No. (%)			
M	12 (26)	36 (27)	.90†
F	34 (74)	97 (73)	
Race/ethnicity, No. (%)			
White	28 (61)	84 (63)	.90†
African American	12 (26)	35 (26)	
Hispanic	6 (13)	14 (11)	
Pulmonary function	75% $\pm$ 26% predicted PEFR (after treatment)	57% $\pm$ 10% predicted base- line FEV ₁	

*Determined by means of *t* test.

†Determined by means of chi-square analysis.

1100 pg/mL]; geometric mean [-1 SD, $+1$ SD) than in those with stable asthma (350 pg/mL [190, 620 pg/mL]; $P = .0008$, Fig 1). Within the acute asthma group, plasma eotaxin levels were significantly higher among patients who did not increase their PEFR by at least 20% of the predicted normal value after emergency department asthma treatment (660 pg/mL [300, 1480 pg/mL], $n = 21$) than among those who improved by 20% or greater after treatment (410 pg/mL [210, 800 pg/mL]; $n = 22$, $P = .04$, Table II).

Trends. Additional data from these analyses were con-

TABLE II. Eotaxin values by features of patients presenting for acute asthma treatment

Variable	Log eotaxin level $\pm$ SD	<i>P</i> value	Geometric mean, pg/mL (-1 SD, $+1$ SD)
Peak flow improvement after emergency treatment			
<20%	2.82 $\pm$ 0.35	.04	660 (300, 1480)
$\geq 20\%$	2.61 $\pm$ 0.29		410 (210, 800)
Systemic steroid therapy on presentation			
Yes ($n = 8$)	2.90 $\pm$ 0.25	.07	790 (450, 1410)
No ($n = 38$)	2.68 $\pm$ 0.33		480 (220, 1020)
Admission to hospital			
Yes ($n = 11$)	2.87 $\pm$ 0.31	.08	740 (360, 1510)
No ($n = 35$)	2.67 $\pm$ 0.32		470 (220, 980)

Log = base 10 logarithm.

sistent with an association between plasma eotaxin levels and asthma severity. Plasma eotaxin levels were higher in the 11 patients who were subsequently admitted to the hospital (740 pg/mL [360, 1510 pg/mL]) than in the 35 patients who were discharged after emergency department treatment (470 pg/mL [220, 980 pg/mL]; $P = .08$). Plasma eotaxin levels in patients undergoing asthma exacerbation who were taking oral steroids on presentation tended to be higher (790 pg/mL [450, 1410 pg/mL], $n = 8$) than those of patients with acute asthma not taking oral steroids (480 pg/mL [220, 1020 pg/mL]; $n = 38$, $P = .07$, Table II).

To better understand the temporal relationship between plasma eotaxin levels and asthma exacerbations, we segregated our population into categories on the basis of duration of acute symptoms. We found a unimodal distribution, with the highest levels in patients who reported symptoms for 4 to 12 hours and lower levels in patients with symptoms of less than 3 hours' or more than 1 weeks' duration (Fig 2).

DISCUSSION

This study documented that plasma eotaxin levels were increased in spontaneously occurring asthma exacerbations. We documented that plasma eotaxin levels were significantly higher in patients seeking emergency treatment for asthma symptoms than in control subjects with stable asthma matched for age, ethnicity, and sex and that plasma eotaxin levels were inversely associated with the extent of β -agonist bronchodilator response. Plasma eotaxin levels were lower among patients with acute asthma who responded to bronchodilator treatment with a PEFR increase of 20% or greater of their predicted normal value than among those who failed to improve by this amount. When the study cohort was stratified by intensity of therapy, we found a trend toward higher

eotaxin levels in asthmatic subjects who were taking oral steroids than in those who were not and in those admitted to the hospital than in those discharged after emergency treatment. All of these findings are consistent with a direct association between eotaxin expression and acute asthma. The nature of our study design does not allow us to distinguish whether eotaxin is involved directly as a mediator or as a biomarker of the acute asthmatic response.

Median plasma eotaxin levels in the 46 asthmatic subjects presenting to the emergency department for acute treatment were higher than those previously reported for a large cohort ($n = 515$) of subjects with stable asthma (480 pg/mL [300-970 pg/mL] vs 355 pg/mL [240-540 pg/mL]; median [interquartile range]). The levels measured in 133 subjects with stable asthma (330 pg/mL [240-470 pg/mL]) were similar to those reported in subjects with stable asthma and are greater than those reported for a population of 519 nonatopic nonasthmatic subjects (290 pg/mL [200-380 pg/mL]).¹⁴ This prior study demonstrated that plasma eotaxin levels are inversely related to lung function (FEV_1) in patients with chronic stable asthma. The current investigation links plasma eotaxin levels directly to the clinical acuity or severity of asthma exacerbations. Because eotaxin levels increase as lung function declines in patients with chronic stable asthma,¹⁴ we cannot attribute the elevated eotaxin levels observed in our patients to differences in lung function because lung function was not worse in our patients than in our control subjects.

Studies in cell systems and in animal models suggest that under some circumstances eotaxin mobilization is limited by corticosteroid exposure.¹⁰ Although not excluding a separate effect of steroid therapy on eotaxin levels, suppressive effects of steroid therapy cannot explain higher plasma eotaxin levels in our steroid-treated patients. The current finding that acutely ill patients who report taking oral or inhaled steroids tend to have higher plasma eotaxin levels demonstrates that in some asthmatic subjects steroids do not inhibit the robust expression of eotaxin. Moreover, higher eotaxin levels in steroid-treated patients with acute asthma is consistent with our main hypothesis that eotaxin levels are directly related to asthma severity and leads to the speculation that therapeutic strategies aimed at blocking the effects of eotaxin by inhibiting its receptor (CCR3) might benefit some symptomatic, corticosteroid-treated asthmatic subjects.

Evidence from this and other studies links plasma eotaxin levels to asthma severity^{13,14} and is consistent with eotaxin's role as a mediator of asthma exacerbations. We found the relationship between the reported duration of asthma symptoms and plasma eotaxin levels to be of interest. Eotaxin levels in bronchoalveolar lavage fluid peak 4 to 12 hours after transient bronchoconstriction is induced by inhalational allergen challenge.¹¹ In acutely ill asthmatic subjects, plasma eotaxin levels were highest when symptoms had been present for 4 to 12 hours (Fig 2), an observation indicating a similar time

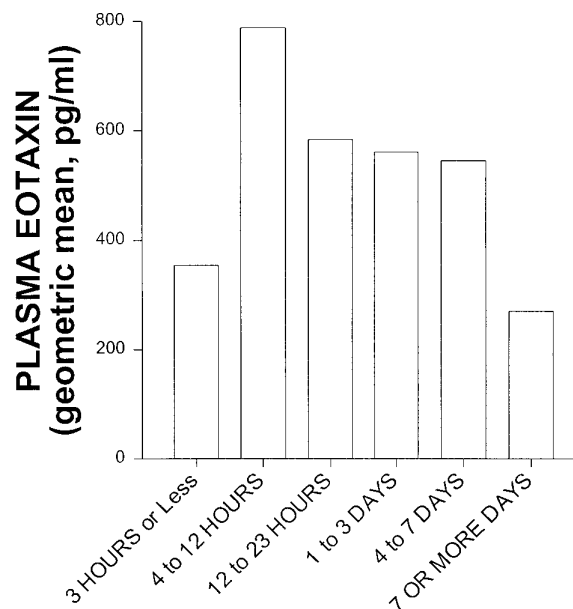


FIG 2. Plasma eotaxin levels of asthmatic subjects presenting for emergency treatment grouped by the duration of acute asthma symptoms before presentation. Data are presented as geometric means.

course of onset of eotaxin expression in these settings. Plasma eotaxin levels were elevated even when asthma symptoms had been present for 4 to 7 days; this finding contrasts with laboratory challenge models in which eotaxin levels in bronchoalveolar lavage fluid are only modestly elevated 24 hours after challenge.¹¹ We speculate that in patients with spontaneous asthma exacerbations the more severe and prolonged reduction in lung function is associated with more robust eotaxin expression in the airways.

At least 2 limitations of this investigation must be considered in interpreting these results. First, the study was designed as a cross-sectional comparison of 2 separate groups of asthmatic subjects. The issue of whether eotaxin levels are chronically elevated in asthmatic subjects who are prone to exacerbations or are elevated during exacerbations cannot be conclusively addressed by this study. Second, the numbers of patients with asthma exacerbations available for study did not allow the observed increase in eotaxin levels for the severity measures to reach statistical significance. These issues could be resolved in a larger study with a longitudinal cohort design or with a CCR3 receptor antagonist. Moreover, the overlap in plasma eotaxin levels observed in our patients compared with the control subjects is consistent with the known biology of mediators in asthma and supports the concept that eotaxin is one of many substances mediating asthma severity.

In summary, we found higher plasma eotaxin levels in patients with acute asthma than in age-, ethnicity-, and sex-matched asthmatic control subjects not experiencing an asthma exacerbation. Acutely ill asthmatic subjects

who did not respond to emergency treatment had higher eotaxin levels than did those who responded, and eotaxin levels tended to be higher in those admitted to the hospital than in those discharged to home. Thus we conclude that eotaxin expression is directly associated with spontaneously occurring asthma.

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