Airway immunopathology of asthma with exercise-induced bronchoconstriction

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Background: Exercise-induced bronchoconstriction (EIB) is a common cause of symptoms in a subgroup of asthmatic subjects. The pathobiology that makes this group of asthmatic subjects susceptible to bronchoconstriction after a brief period of exercise remains poorly understood.

Objective: We sought to determine whether there are differences in lower airway inflammation and production of cytokines and eicosanoids between asthmatic subjects with and without EIB.

Methods: Two distinct groups of asthmatic subjects based on *a priori* definitions were identified, one with moderate-to-severe EIB and the other without significant bronchoconstriction after exercise challenge. Both groups met the definition of asthma on the basis of bronchodilator response, bronchial hyperresponsiveness, or both. A comparative immunopathology study was conducted by using induced sputum to identify differences in lower airway inflammation and production of cytokines and eicosanoids.

Results: The groups had similar baseline lung function and bronchodilator response and did not have any asthma exacerbations within the prior year. The concentration of columnar epithelial cells was markedly higher in the group with EIB (1.4×10^5 vs 2.9×10^4 cells/mL, $P = .01$). The concentration of eosinophils was higher in the group with EIB (3.6×10^4 vs 4.9×10^3 cells/mL $P = .04$). Cysteinyl leukotrienes (CysLTs; 727.7 vs 151.9 pg/mL, $P = .01$) and the ratio of CysLTs to prostaglandin E_2 (1.85 vs 1.04, $P = .002$) in the airways were higher in the group with EIB.

Conclusion: Injury to the airway epithelium, overexpression of CysLTs, relative underproduction of prostaglandin E_2, and greater airway eosinophilia are distinctive immunopathologic features of asthma with EIB. (J Allergy Clin Immunol 2005;116:586-93.)

Key words: *Asthma, exercise-induced bronchoconstriction, epithelial cell, leukotriene, prostaglandin, eosinophil, mast cell*

Asthma is a complex syndrome with a number of clinical phenotypes.[1] Exercise-induced bronchoconstriction (EIB) is a highly prevalent but discrete clinical phenotype that shares common features with other measures of indirect bronchial hyperresponsiveness (BHR).[2] Among triggers of indirect BHR, exercise is particularly important because it generally cannot be avoided. Recent studies indicate that EIB occurs in less than half of asthmatic subjects tested with a standardized exercise challenge.[3,4] Compared with other features of asthma, EIB has distinct pathophysiology. There is no relationship between baseline lung function and severity of EIB.[4] Only a weak relationship exists between direct BHR and severity of EIB.[5,6] The presence of EIB in children might precede the development of other features of asthma, representing an early stage of the disease.[7] Some asthmatic subjects susceptible to EIB have severe bronchoconstriction and hypoxemia after a brief period of exercise.[8] Furthermore, asthma triggered during sports is a common cause of sports-related deaths in children.[9]

The pathobiology that makes this group of asthmatic subjects susceptible to bronchoconstriction after a short period of exercise remains poorly understood. Because the exercise challenge test used to identify EIB is the maximum stimulus for EIB,[10] asthmatic subjects with and without EIB can be readily identified by threshold responses to a standardized exercise challenge test.[10] The stimulus for EIB involves drying and cooling of the intrathoracic airways as a result of increased ventilation during exercise.[11] After exercise challenge in human subjects with EIB, increased levels of cysteinyl leukotrienes (CysLTs; leukotriene E_4)[12,13] are detected in the urine, although these findings have not been seen in all studies.[14] In a canine model of EIB, epithelial disruption occurs during dry air challenge, which is correlated with the amount of bronchoconstriction and release of CysLTs into the airways.[15,16] Airway eosinophilia is correlated with the severity of EIB in asthmatic subjects with EIB.[6] To determine whether there are differences in lower airway inflammation and production of cytokines and

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Abbreviations used
AUC: Area under the FEV_1-time curve
BHR: Bronchial hyperresponsiveness
CysLT: Cysteinyl leukotriene
EIB: Exercise-induced bronchosontriction
PG: Prostaglandin

eicosanoids between asthmatic subjects with and without EIB, we identified 2 distinct groups of asthmatic subjects on the basis of *a priori* definitions, one with moderate-to-severe EIB and the other without significant bronchoconstriction after exercise challenge. Both groups met the definition of asthma on the basis of bronchodilator response, BHR, or both.[17] We conducted a comparative immunopathology study by using induced sputum. The primary objective of the study was to determine whether asthmatic subjects with EIB have epithelial injury, leading to airway eosinophilia and release of CysLTs into airway fluid.

METHODS

Subjects

The University of Washington Institutional Review Board approved the study protocol, and written informed consent was obtained from all participants. Subjects 18 to 59 years of age were recruited who had a physician's diagnosis of asthma for 1 year or longer and used only an inhaled β_2-agonist for asthma treatment. In accordance with *a priori* definitions, asthmatic subjects with EIB (EIB^+ group) were identified with a 30% or greater decrease in FEV_1 after exercise challenge, and asthmatic control subjects without EIB (EIB^- group) were identified with a 7% or less decrease in FEV_1 after exercise challenge. A diagnosis of asthma was established in the EIB^- group on the basis of either a change in FEV_1 of 12% or greater after the administration of a β_2-agonist or a methacholine PC_{20} of 8 mg/mL or less. Potential participants were excluded if baseline FEV_1 was 65% of predicted value or less, if there was history of smoking cigarettes within the prior year or a 7 pack-years or longer smoking history, if the patient was treated for acute asthma within the prior month, if the patient was hospitalized for asthma within the prior 3 months, or if the patient had a history of life-threatening asthma. Participants were excluded if they had used an inhaled corticosteroid, leukotriene modifier, long-acting antihistamine, cromone, or long-acting β_2-agonist in the 30 days before the study.

Study protocol

The first visit consisted of a physical examination, spirometry, and exercise challenge to determine eligibility for the study. Participants with a 30% or greater maximum decrease in FEV_1 after exercise challenge were enrolled in the EIB^+ group. Participants with a 7% or smaller maximum decrease in FEV_1 after exercise challenge were eligible for inclusion in the EIB^- group. On the second visit, conducted at the same time of day 4 to 10 days later, all participants had spirometry before and 15 minutes after administration of 180 μg of albuterol through a metered-dose inhaler, followed by induced sputum. Participants in the EIB^- group with less than a 12% improvement in FEV_1 after the administration of albuterol had a third study visit for a methacholine challenge 4 to 10 days later. Subjects were asked not to exercise, not to use short-acting antihistamines for 48 hours, and not to use β_2-agonists and caffeinated beverages for 6 hours before each study visit. All participants in the EIB^+ group were enrolled in a subsequent study.[18]

Spirometry, exercise, and methacholine challenge

Spirometry, exercise, and methacholine challenges were conducted in accordance with American Thoracic Society standards.[10,19] Exercise challenge was performed on a motorized treadmill such that each subject sustained 85% or more of their maximum heart rate for the final 6 minutes of exercise.[10] Subjects wore nose clips and breathed dry air (0% relative humidity, 22°C) delivered from a weather balloon reservoir through a 1-way valve (Hans Rudolph, Kansas City, Mo) during exercise. Spirometry was conducted 20 and 5 minutes before each exercise challenge and repeated at 0, 3, 6, 10, 15, and 30 minutes after the end of exercise. The better of at least 2 FEV_1 maneuvers within 5% of each other was recorded at each time point. Methacholine challenge was conducted with a dosimeter.[10]

Sputum induction

Induced sputum was conducted with 3% hypertonic saline administered through an ultrasonic nebulizer (DeVilbiss, Somerset, Pa), as previously described.[20] At 2-minute intervals, subjects were asked to clear saliva from their mouth and then expectorate sputum. Sputum was collected over 12 minutes and was pooled into a single sample container. The induced sputum was placed on ice immediately and processed within 30 minutes of collection. Samples were coded with a subject number, visit number, and date. The link between the clinical characteristics of the participants and the coded label was maintained in a separate file by the principal investigator. Total and differential cell counts were performed by an investigator (MWM) who was blinded to the clinical characteristics of each participant. The levels of histamine, CysLTs, leukotriene B_4, prostaglandin (PG) E_2, IL-4, IL-5, IL-8, IL-13, TNF-α, vascular endothelial growth factor, and RANTES were determined in induced sputum supernatants. Details of induced sputum processing and analysis are presented in the Online Repository in the online version of this article at www.mosby.com/jaci.

Statistical analysis

The sample size was based on the percentage of eosinophils because of the relationship between the percentage of eosinophils in induced sputum and the severity of EIB.[6] The sample size in this study was selected to detect a 2% difference in the percentage of eosinophils with 95% power on the basis of data from Crimi et al.[21] The characteristics of the study participants were compared with unpaired *t* tests for continuous variables and χ^2 tests for categoric variables. The area under the FEV_1-time curve (AUC)[22] quantified the severity of EIB over a 30-minute period after exercise (AUC_{30}). Comparison of the severity of EIB between the groups was made with an unpaired *t* test. The relationship between lung function and bronchodilator response and the severity of EIB was assessed within each group with the Pearson correlation coefficient. The medians of differential cell counts and concentrations of cellular constituents and inflammatory mediators were compared between different groups with the Mann-Whitney *U* test. On the basis of the study by Szefler et al,[23] indicating that age might be a factor in the response to the $CysLT_1$ antagonists, a regression analysis (see the Online Repository in the online version of this article at www.mosby.com/jaci) was also performed to account for the possible effects of age and sex on the levels of cellular constituents and inflammatory mediators in induced sputum.