Published in final edited form as:

J Allergy Clin Immunol. 2007 May ; 119(5): 1092–1098.

Role of MUC5AC in the pathogenesis of exercise-induced bronchoconstriction

Teal S. Hallstrand, MD, MPH^a, Jason S. Debley, MD, MPH^b, Federico M. Farin, MD^c, and William R. Henderson Jr, MD^a

^aDepartment of Medicine, University of Washington, Seattle.

^bDepartment of Pediatrics, University of Washington, Seattle.

^cDepartment of Environmental Health, University of Washington, Seattle.

Abstract

Background: The pathogenesis of exercise-induced bronchoconstriction (EIB) involves the release of mediators from several airway cells in response to exercise challenge, but the mechanism leading to airflow obstruction during EIB is incompletely understood.

Objective: To evaluate the role of secreted mucin in the pathogenesis of EIB.

Methods: Induced sputum was collected at baseline and 30 minutes after exercise challenge in patients with asthma with EIB. The expression of gel-forming mucins and epidermal growth factor receptor ligands were assessed by quantitative polymerase chain reaction. Secreted mucin 5AC (MUC5AC), the eicosanoids cysteinyl leukotrienes (cysLTs) and 15S-hydroxyeicosatetraenoic acid (15S-HETE), and tachykinins neurokinin A (NKA) and substance P (SP) were measured in induced sputum supernatant.

Results: Among the gel-forming mucins, MUC5AC was expressed at the highest level. The gene expression of MUC5AC increased after exercise challenge compared with baseline and was associated with EIB severity by regression analysis. The relative levels of MUC5AC in induced sputum increased from a geometric mean of 9.5 at baseline to 18.4 postexercise challenge. Associations between the levels of MUC5AC and cysLTs and between the levels of cysLTs and NKA postexercise challenge were identified by regression analysis.

Conclusions: These data indicate that (1) the predominant gel-forming mucin expressed in induced sputum of patients with asthma with EIB is MUC5AC; (2) an increase in MUC5AC gene expression and release of MUC5AC protein occurs after exercise challenge; and (3) MUC5AC release may occur through the cysLT-associated activation of sensory airway nerves.

Keywords

Asthma; exercise-induced bronchoconstriction; epithelial cell; goblet cell; mucin

Exercise-induced bronchoconstriction (EIB) is triggered by inhalation of cold and/or dry air in 40-50% of patients with asthma¹ and approximately 10% to 20% of all adolescents.^{2,3} The pathophysiology of EIB is complex, involving the release of eicosanoids, such as cysteinyl

Reprint requests: Teal S. Hallstrand, MD, MPH, Division of Pulmonary and Critical Care, Department of Medicine, University of Washington, Box 356522, Seattle, WA 98195-6522. E-mail: tealh@u.washington.edu.

Disclosure of potential conflict of interest: T. S. Hallstrand has consulting arrangements with Merck and GlaxoSmithKline; has received grant support from Merck; and is on the speakers' bureau for Merck. W. R. Henderson has consulting arrangements with Alza Corporation, Icos, and Critical Therapeutics; has received grant support from Sepracor and Merck; and is on the speakers' bureau for Critical Therapeutics and Sepracor. The rest of the authors have declared that they have no conflict of interest.

leukotrienes (cysLTs) and prostaglandin D₂ (PGD₂), into the airways from mast cells, eosinophils, and other airway cells.^{4,5} The mechanism by which the release of these products into the airways, after exercise challenge, leads to airflow obstruction is incompletely understood.

Emerging evidence indicates that injury to the airway epithelium is a key susceptibility factor for EIB.^{6,7} One consequence of epithelial injury is replacement of ciliated epithelial cells by mucin secreting cells, primarily goblet cells.⁸ The secreted or gel-forming mucins, MUC2, MUC5AC, MUC5B, and MUC6, are highly glycosylated proteins that constitute a major component of the gel layer of the airway surface liquid.⁹ The composition of this gel layer has major effects on the hydration of the airways and the viscoelastic properties of airway surface liquid, which contribute to alteration in lung function and airway clearance.⁸ Alterations in the epithelial mucin secretory phenotype occur largely through the epidermal growth factor (EGF) receptor signaling cascade.¹⁰⁻¹²

Although no human studies exist, dry air hypernea in a canine model of EIB and hyperosmolar stimuli in *in vitro* epithelial cell models initiate mucin secretion, which implicates mucin release in the pathophysiology of EIB.¹³⁻¹⁵ 5-lipoxygenase (5-LO) products, such as cysLTs and other eicosanoids, including 15S-hydroxyeicosatetraenoic acid (15S-HETE), are potent mucin secretagogues in model systems,^{16,17} and their effect on mucin secretion is mediated in part through the release of tachykinins from sensory airway nerves.^{18,19}

To assess the role of airway mucins in the pathogenesis of EIB, we collected induced sputum from patients with asthma with EIB at baseline and on a separate day 30 minutes after dry air exercise challenge. We measured the gene expression of the major gel-forming mucins and EGF receptor ligands in induced sputum cells, assessed the release of the major gel-forming mucin MUC5AC in response to exercise challenge, and determined the levels of selected eicosanoids cysLTs and 15S-HETE and the levels of tachykinins neurokinin A (NKA) and substance P (SP) in induced sputum supernatant. Our goals were to determine (1) which gel-forming mucins and EGF receptor ligands predominate in induced sputum of patients with asthma with EIB; (2) if MUC5AC released into the airways during EIB contributes to the pathophysiology of airflow obstruction; and (3) if the release of MUC5AC is associated with the release of the selected eicosanoids and/ or tachykinins into the airways.

METHODS

Subjects and study protocol

A detailed description of these methods can be found in this article's Online Repository at www.jacionline.org. The University of Washington Institutional Review Board approved the study protocol, and written informed consent was obtained from all participants. Samples for this study were obtained from the same cohort of 25 patients with asthma with EIB who were 12-59 years of age, had a physician diagnosis of asthma for ≥ 1 year, used only an inhaled β_2 -agonist for asthma treatment, and had $\geq 15\%$ fall in FEV₁ after exercise challenge.⁴ Induced sputum samples were obtained at baseline and then on a separate day 30 minutes after exercise challenge. Albuterol (180 μ g via a metered dose inhaler) was administered 15 minutes before sputum induction. Twenty-two subjects had induced sputum samples that were available for analysis of mucin gene expression and/or secreted mucin from both the baseline and the post-exercise visits. The cell pellet from induced sputum was available for gene expression analysis at both baseline and postexercise in 11 subjects, and the induced sputum supernatant was available for analysis of secreted mucin in 19 subjects. The levels of tachykinins NKA and SP were measured in 10 representative paired samples at baseline and postexercise challenge.

Mucin and EGF receptor ligand gene expression

Induced sputum samples were immediately placed on ice and processed within 30 minutes of collection. The induced sputum cell pellet was resuspended in RLT buffer (Qiagen, Valencia, Calif) with β -mercaptoethanol, mechanically lysed, and stored at -80°C . Total RNA was extracted from the lysed cell pellet using the RNeasy protocol (Qiagen). cDNA was prepared from 100 ng of total RNA using oligo(dT) primers (Invitrogen, Carlsbad, Calif). Primer and probe sets for the gel-forming mucins MUC2, MUC5AC, MUC5B, and MUC6 and the EGF receptor ligands TGF- α , EGF, heparin binding (HB)-EGF, amphiregulin, and TNF- α converting enzyme (TACE)/ADAM17 were designed using ABI Primer Express version 1.5 software (Applied Biosystems, Foster City, Calif).

Measurement of secreted MUC5AC

Levels of secreted MUC5AC were measured using a sandwich enzyme-linked immunosorbent assay consisting of a monoclonal anti-MUC5AC capture antibody (I-13M1, Neomarkers, Fremont, Calif) and a monoclonal anti-MUC5AC detection antibody (45M1, Neomarkers) labeled with biotin. Because a MUC5AC standard is not commercially available, a lysate of the human tumorigenic lung epithelial cell line A549 was used in preliminary studies. The effect of dithiothreitol (DTT) on the measured level of MUC5AC was assessed by comparing the relative levels of a series of dilutions of the A549 cell lysate with or without DTT. Based on these results, the best dynamic range was achieved using a standard curve created by a series of dilutions of an induced sputum sample after dialysis to remove the DTT.

Measurement of eicosanoids and tachykinins

Induced sputum supernatant for eicosanoid analysis was treated with 4 volumes of methanol and stored at -80°C . The levels of cysLTs and 15S-HETE were measured by enzyme immunoassay after solid phase extraction on an Oasis HLB column (Waters, Milford, Mass). Induced sputum supernatant for tachykinin analysis was stored at -80°C in the presence of protease inhibitors. The levels of NKA and SP were measured after solid-phase extraction on a C-18 column.²⁰

Statistical analysis

The characteristics of the study subjects were described according to the mean and standard deviation. The severity of EIB was quantified by the maximum fall in FEV₁ after exercise challenge and the area under the FEV₁ time curve over the first 30 minutes after exercise challenge (AUC₃₀).²¹ A paired *t* test was used to compare the ratio of gene expression of the gel-forming mucins relative to β -actin between baseline and postexercise. A paired *t* test was also used to compare the levels of MUC5AC, eicosanoids, and tachykinins in induced sputum at baseline and postexercise after log transformation. The relationships between the levels of secreted MUC5AC and pulmonary function and symptom parameters were assessed by linear regression. The relationships among MUC5AC, eicosanoids, and tachykinins were assessed by linear regression after log transformation. *P* values $\leq .05$ were considered statistically significant, and values between .05 and .10 were considered trends.

RESULTS

Subject characteristics

The study subjects had mild-to-moderate asthma and at least a 15% fall in FEV₁ after a standardized exercise challenge to induce EIB on the screening visit. The characteristics of the study subjects are presented in Table I. The severity of EIB ranged from a maximal fall in FEV₁ of 15% to 47% after exercise challenge. The average time between the baseline induced

sputum and the induced sputum conducted after exercise challenge was 9.5 days and ranged from 4 to 18 days.

Distribution of gel-forming mucins

Genes for the gel-forming mucins, MUC2, MUC5AC, and MUC5B were expressed in induced sputum cells at baseline, whereas MUC6 could not be detected. After normalization to the level of β -actin, MUC5AC was the predominant gel-forming mucin expressed in patients with asthma with EIB (Fig 1, A). TACE and the EGF ligands EGF, HB-EGF, amphiregulin, and TGF- α were detectable at baseline. The EGF ligands HB-EGF, amphiregulin, and TGF- α were expressed at higher levels than EGF (Fig 1, B).

The expression of MUC5AC at baseline was associated with the baseline forced expiratory flow at 25% to 75% of FVC ($R^2 = .38$, $P = .04$). There was also a trend between the expression of MUC5AC and the FEV₁ to FVC ratio ($R^2 = .34$, $P = .06$). The expression of MUC5AC at baseline was not associated with baseline FEV₁ ($R^2 = .23$, $P = .14$) or the severity of EIB measured by the AUC₃₀ ($R^2 = .21$, $P = .15$).

Effects of exercise challenge on mucin gene expression

Exercise challenge had no effect on the expression of β -actin in induced sputum cells between the baseline and the postexercise samples (.59 vs .47, $P = .46$). The expression of MUC5AC increased 30 minutes after exercise challenge as compared with the baseline value (Fig 2, A). The increase in MUC5AC gene expression after exercise challenge was associated with the severity of EIB by regression analysis (Fig 2, B). The expression of MUC2 (5.0×10^{-6} vs 7.4×10^{-6} , $P = .63$) and MUC5B (5.6×10^{-5} vs 4.2×10^{-5} , $P = .46$) was not significantly altered by exercise challenge. Changes in MUC2 and MUC5B gene expression after exercise challenge were not associated with the severity of EIB. No significant changes occurred in the expression of EGF ligands or TACE in induced sputum between baseline and postexercise (P values = .25-.89).

Effects of exercise challenge on secreted MUC5AC

The levels of secreted MUC5AC were measured on a relative scale using a standard curve of an induced sputum supernatant sample dialyzed against phosphate-buffered saline using a 3.5-kd membrane. The highest value on the standard curve used to measure MUC5AC was set at a value of 1000. The level of secreted MUC5AC in the baseline induced sputum was not associated with baseline FEV₁, FEV₁ to FVC ratio, forced expiratory flow at 25% to 75% of FVC, or severity of EIB (P values = .51-.89).

The relative level of MUC5AC increased from a geometric mean of 21.4 units at baseline to a geometric mean of 35.6 units after exercise challenge ($n = 19$, $P = .03$). Three subjects had baseline MUC5AC levels that were outliers ≥ 2.5 SD above the other baseline values and could only be measured at 1:100 dilution of the sputum supernatant. After exclusion of these 3 outliers, the results showed that an increase occurred in the MUC5AC level from a geometric mean of 9.5 units at baseline to a geometric mean of 18.4 units after exercise challenge (Fig 3; $n = 16$, $P = .007$). Although the level of secreted MUC5AC in the induced sputum after exercise challenge was not associated with the severity of EIB measured by the maximum fall in FEV₁ after exercise or the AUC₃₀, trends were noted between the level of secreted MUC5AC and the decline in FEV₁ 15 minutes after exercise challenge ($R^2 = .18$, $P = .10$) and symptoms 15 minutes after exercise challenge on the Borg scale ($R^2 = .22$, $P = .07$).

Relationship between secreted MUC5AC and airway eicosanoids and tachykinins

The concentration of cysLTs in induced sputum after exercise challenge was associated with the level of secreted MUC5AC (Fig 4, A). The levels of 15S-HETE between baseline and postexercise samples were not significantly different (geometric mean 3248.3 vs 4499.3 pg/mL, $P = .18$). The concentration of 15S-HETE after exercise challenge was not associated with the level of secreted MUC5AC (Fig 4, B).

No difference was observed between baseline and postexercise levels of NKA (geometric mean 42.3 vs 56.1 pg/mL, $P = .13$), or SP (geometric mean 6.2 vs 6.1 pg/mL, $P = .91$). The level of NKA was associated with the level of cysLTs after exercise challenge (Fig 5). The level of SP was not associated with the level of cysLTs after exercise challenge ($R^2 = .11$, $P = .34$). The levels of secreted MUC5AC after exercise challenge were not associated with the levels of NKA ($R^2 = .23$, $P = .16$), or SP ($R^2 = .26$, $P = .13$).

DISCUSSION

The pathophysiology of EIB is complex, involving the release of multiple bronchoconstrictive products from mast cells, eosinophils, and other airway cells.^{4,5} The mechanism by which the release of these products into the airways leads to airflow obstruction after exercise challenge is incompletely understood. The release of mucins into the airway significantly contributes to airflow obstruction in asthma.⁸ In this study, we determine that, at the level of gene expression, the gel-forming mucin MUC 5AC predominates in induced sputum cells of patients with asthma with EIB, and that there is an increase in MUC5AC gene expression and release of MUC5AC into the airways in response to exercise challenge. Furthermore, we identified associations between the levels of MUC5AC and cysLTs in the airways, and the levels of cysLTs and NKA in the airways, showing that these are closely associated events that implicate a mechanism in which MUC5AC release occurs via the cysLT-mediated activation of sensory airway nerves.

Under stable “baseline” conditions, significant heterogeneity occurs in the amount, type, and size of mucins between sputa obtained from subjects with and without airways disease.²² In a study of induced sputum from subjects with asthma, MUC5AC glycoprotein predominated, whereas smaller amounts of MUC5B and little MUC2 were present.²³ We found that MUC5AC was the predominant gel-forming mucin at the level of gene expression and that MUC5AC protein was present in large quantities in induced sputum. These findings are consistent with an endobronchial biopsy study in mild-to-moderate asthma, which demonstrates that MUC5AC gene expression predominates and that the number of goblet cells are increased.²⁴

Remodeling of the airway epithelium to a secretory phenotype is implicated in the pathophysiology of asthma.⁸ This alteration in the epithelial phenotype may represent the response to injury of the airway epithelium that is implicated in the development of EIB.^{25, 26} Elite athletes training in cold and dry air develop EIB at a high rate and have an abnormal epithelium infiltrated by inflammatory cells.²⁶ In canines, repeated bouts of dry air hyperpnea lead to disruption and inflammation of the epithelium and increased susceptibility to hyperpnea-induced bronchoconstriction.²⁵ We have found that patients with asthma susceptible to EIB have higher levels of columnar epithelial cells in induced sputum than patients with asthma without EIB,⁷ and that the concentration of epithelial cells in induced sputum is associated with the severity of EIB.⁴ The EGF signaling pathway plays a key role in the development of the mucin secreting epithelial phenotype *in vitro* indicated by inhibition of mucin gene expression and epithelial proliferation by an EGF receptor tyrosine kinase inhibitor.¹⁰⁻¹² In these model systems, the EGF receptor ligand that plays a dominant role is TGF- α .¹⁰ We found here that EGF was expressed at low levels in the airways, but that the

EGF ligands TGF- α , AREG, and HB-EGF were all expressed at higher levels, which indicates that expression of airway mucins in EIB may be mediated through 1 of these ligands.

Exercise initiates acute bronchoconstriction in patients with asthma who are susceptible to EIB, but it is not known if mucin release contributes to the pathophysiology of EIB. We found that exercise challenge resulted in a significant release of MUC5AC in the airways. As MUC5AC is predominantly a goblet cell product, these results imply that goblet cell degranulation occurs during EIB. Data in the canine model of EIB are supportive of this finding, which indicates that dry air hyperpnea delivered through a bronchoscope into the lower airways initiates goblet cell degranulation.¹³ Interestingly, allergen challenge in subjects with mild-to-moderate asthma did not initiate the release of mucin,²⁷ although in the current study, we used specific MUC5AC monoclonal antibodies to measure MUC5AC release, whereas the prior study used a mucin-like glycoprotein antibody. Although the release of MUC5AC in the current study was not associated with the severity of bronchoconstriction immediately after exercise challenge, notable trends were identified between the level of MUC5AC and the level of persistent airflow obstruction and symptoms 15 minutes after exercise challenge, which suggests that persistent airflow obstruction in EIB may be mediated in part by the release of airway mucins. Recent evidence showing that asthma triggered during sports is the leading cause of nontraumatic death in adolescent athletes²⁸ raises the concern that mucus obstruction of the airways implicated in fatal asthma could play a role in these exacerbations triggered during sports. Because we did not enroll a group of subjects without EIB in the current study, we cannot exclude the possibility that mucin is released in the absence of airflow obstruction after exercise challenge. In addition, the hypertonic saline used for the induced sputum could also trigger mucin release, but it would not account for the increase in MUC5AC identified in the paired samples analyzed in this study comparing baseline to postexercise levels of MUC5AC.

The mechanism initiating the release of mucin into the airways could be directly through alterations in the osmolarity of the airway surface liquid, as has been identified in some *in vitro* studies^{14,15,29} or mediated through the release of a mediator into the airways. Regression analysis in the current study indicates that the release of MUC5AC and cysLTs are strongly associated events, which suggests that mucin release may be mediated via the release of cysLTs into the airways. Products of 5-LO including the cysLTs are implicated in the release of mucins into the airways in a murine model of asthma.^{16,30} The release of 15S-HETE can occur directly from the airway epithelium and serves as a strong mucin secretagogue,¹⁷ but it was not as strongly associated with the release of MUC5AC in this study as the cysLTs. As several eicosanoid products including cysLTs, PGD₂, PGF_{2 α} , and thromboxane B₂ are implicated in EIB,^{4,5,31} the release of airway mucins may occur as the result of combined effects from these products released from several cells in the airways.

Evidence in animal models indicates that the mechanism of hyperpnea-induced bronchoconstriction involves the activation of sensory nerve C-fibers, which initiate the release of tachykinins via retrograde axonal transmission.^{18,19} Three-dimensional mapping indicates that these C-fibers enter the epithelium and wrap around goblet cells.³² Degranulation of goblet cells is triggered by the activation of these nerve fibers.³³ In the guinea pig model of hyperpnea-induced bronchoconstriction, a NK2 receptor antagonist inhibited bronchoconstriction without altering the LT levels, whereas either a cysLTR₁ antagonist or a 5-LO inhibitor significantly reduced bronchoconstriction and the release of SP.³⁴ Similar results were identified in the dog model of hyperpnea-induced bronchoconstriction using a combination of NK1 and NK2 antagonists.³⁵ In the current study, we found that the release of cysLTs and NKA were strongly associated events, which suggests that the release of NKA may occur via the cysLT-associated activation of sensory nerves. These data are consistent with the findings in human subjects with asthma that a cysLTR₁ antagonist reduces the severity of NKA-induced and bradykinin-

induced bronchoconstriction.^{36,37} Several eicosanoids, including 15S-HETE, directly activate sensory nerves,³⁸ whereas cysLTs such as LTD₄ potentiate C-fiber-mediated sensory nerve activation.³⁹ Thus, activation of sensory nerves could occur as a consequence of a direct-acting stimulus such as a shift in airway osmolarity that would further activate the release of mediators from inflammatory cells such as mast cells.⁴⁰ Our data implicate NKA rather than SP in the pathogenesis of EIB. SP has the greatest affinity for the NK1 receptor, whereas NKA has the greatest affinity for the NK2 receptor.⁴¹ Predominant expression of the NK2 receptor in human subjects with asthma rather than the NK1 receptor⁴² may explain why an NK1 receptor antagonist only partially inhibited EIB or bronchoconstriction induced by hypertonic saline.^{43,44}

In summary, we found that at the level of gene expression MUC5AC is the major gel-forming mucin expressed in induced sputum cells of patients with asthma with EIB, and that the EGF receptor ligands that predominate in EIB are TGF- α , HB-EGF, and AREG. Exercise challenge initiated the release of MUC5AC into the airways related to persistent airflow obstruction and post-exercise symptoms. The release of MUC5AC and cysLTs into the airways and the release of cysLTs and NKA are strongly associated events, which suggests that the release of MUC5AC may be mediated by the cysLT-associated activation of sensory airway nerves containing NKA. These results suggest that therapies targeting the release of cysLTs and other eicosanoids and/or the release of tachykinins may prove beneficial for mucus hypersecretion in asthma.

Acknowledgements

We thank Dr Christopher Brightling for guidance on dialysis of induced sputum supernatant and Dr John Sheehan for advice on measuring airway mucins.

Supported by National Institutes of Health grants K23HL04231 (to T.S.H.) and P30ES07033 (to F.M.F.) and an American Lung Association Clinical Research Grant (to T.S.H.).

Abbreviations used

AREG, Amphiregulin; AUC, Area under the FEV₁ time curve; cysLT, Cysteinyl leukotriene; DTT, Dithiothreitol; EGF, Epidermal growth factor; EIB, Exercise-induced bronchoconstriction; HB-EGF, Heparin-binding EGF; LTD₄, Leukotriene D₄; MUC2, Mucin 2; MUC5AC, Mucin 5AC; MUC5B, Mucin 5B; MUC6, Mucin 6; NK, Neurokinin; NKA, Neurokinin A; SP, Substance P; TACE, TNF- α converting enzyme; 15S-HETE, 15S-hydroxyeicosatetraenoic acid; 5-LO, 5-lipoxygenase.

REFERENCES

1. Cabral AL, Conceicao GM, Fonseca-Guedes CH, Martins MA. Exercise-induced bronchospasm in children: effects of asthma severity. *Am J Respir Crit Care Med* 1999;159:1819–23. [PubMed: 10351925]
2. Hallstrand TS, Curtis JR, Koepsell TD, Martin DP, Schoene RB, Sullivan SD, et al. Effectiveness of screening examinations to detect unrecognized exercise-induced bronchoconstriction. *J Pediatr* 2002;141:343–8. [PubMed: 12219053]
3. Ernst P, Demissie K, Joseph L, Locher U, Becklake MR. Socioeconomic status and indicators of asthma in children. *Am J Respir Crit Care Med* 1995;152:570–5. [PubMed: 7633709]
4. Hallstrand TS, Moody MW, Wurfel MM, Schwartz LB, Henderson WR Jr, Aitken ML. Inflammatory basis of exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2005;172:679–86. [PubMed: 15947280]
5. Mickleborough TD, Lindley MR, Ray S. Dietary salt, airway inflammation, and diffusion capacity in exercise-induced asthma. *Med Sci Sports Exerc* 2005;37:904–14. [PubMed: 15947713]

6. Anderson SD, Kippelen P. Exercise-induced bronchoconstriction: pathogenesis. *Curr Allergy Asthma Rep* 2005;5:116–22. [PubMed: 15683611]
7. Hallstrand TS, Moody MW, Aitken ML, Henderson WR Jr. Airway immunopathology of asthma with exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2005;116:586–93. [PubMed: 16159628]
8. Fahy JV. Remodeling of the airway epithelium in asthma. *Am J Respir Crit Care Med* 2001;164:S46–51. [PubMed: 11734466]
9. Thornton DJ, Sheehan JK. From mucins to mucus: toward a more coherent understanding of this essential barrier. *Proc Am Thorac Soc* 2004;1:54–61. [PubMed: 16113413]
10. Booth BW, Adler KB, Bonner JC, Tournier F, Martin LD. Interleukin-13 induces proliferation of human airway epithelial cells in vitro via a mechanism mediated by transforming growth factor- α . *Am J Respir Cell Mol Biol* 2001;25:739–43. [PubMed: 11726400]
11. Shim JJ, Dabbagh K, Ueki IF, Dao-Pick T, Burgel PR, Takeyama K, et al. IL-13 induces mucin production by stimulating epidermal growth factor receptors and by activating neutrophils. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L134–40. [PubMed: 11133503]
12. Takeyama K, Dabbagh K, Lee HM, Agusti C, Lausier JA, Ueki IF, et al. Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci U S A* 1999;96:3081–6. [PubMed: 10077640]
13. Freed AN, Omori C, Schofield BH, Mitzner W. Dry air-induced mucosal cell injury and bronchovascular leakage in canine peripheral airways. *Am J Respir Cell Mol Biol* 1994;11:724–32. [PubMed: 7946400]
14. Peatfield AC, Richardson PS, Wells UM. The effect of airflow on mucus secretion into the trachea of the cat. *J Physiol* 1986;380:429–39. [PubMed: 3612569]
15. Dwyer TM, Farley JM. Mucus glycoconjugate secretion in cool and hypertonic solutions. *Am J Physiol* 1997;272:L1121–5. [PubMed: 9227513]
16. Henderson WR Jr, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, et al. The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* 1996;184:1483–94. [PubMed: 8879219]
17. Marom Z, Shelhamer JH, Sun F, Kaliner M. Human airway monohydroxyeicosatetraenoic acid generation and mucus release. *J Clin Invest* 1983;72:122–7. [PubMed: 6308043]
18. Bloomquist EI, Kream RM. Leukotriene D₄ acts in part to contract guinea pig ileum smooth muscle by releasing substance P. *J Pharmacol Exp Ther* 1987;240:523–8. [PubMed: 2433430]
19. Ishikawa J, Ichinose M, Miura M, Kageyama N, Yamauchi H, Tomaki M, et al. Involvement of endogenous tachykinins in LTD₄-induced airway responses. *Eur Respir J* 1996;9:486–92. [PubMed: 8730008]
20. Fehder WP, Ho WZ, Campbell DE, Tourtellotte WW, Michaels L, Cutilli JR, et al. Development and evaluation of a chromatographic procedure for partial purification of substance P with quantitation by an enzyme immunoassay. *Clin Diagn Lab Immunol* 1998;5:303–7. [PubMed: 9605981]
21. Leff JA, Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 1998;339:147–52. [PubMed: 9664090]
22. Sheehan JK, Thornton DJ, Somerville M, Carlstedt I. Mucin structure. The structure and heterogeneity of respiratory mucus glycoproteins. *Am Rev Respir Dis* 1991;144:S4–9. [PubMed: 1892325]
23. Kirkham S, Sheehan JK, Knight D, Richardson PS, Thornton DJ. Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. *Biochem J* 2002;361:537–46. [PubMed: 11802783]
24. Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517–23. [PubMed: 11179133]
25. Davis MS, Schofield B, Freed AN. Repeated peripheral airway hyperpnea causes inflammation and remodeling in dogs. *Med Sci Sports Exerc* 2003;35:608–16. [PubMed: 12673144]
26. Karjalainen EM, Laitinen A, Sue-Chu M, Altraja A, Bjerner L, Laitinen LA. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 2000;161:2086–91. [PubMed: 10852791]

27. Hays SR, Woodruff PG, Khashayar R, Ferrando RE, Liu J, Fung P, et al. Allergen challenge causes inflammation but not goblet cell degranulation in asthmatic subjects. *J Allergy Clin Immunol* 2001;108:784–90. [PubMed: 11692105]
28. Becker JM, Rogers J, Rossini G, Mirchandani H, D'Alonzo GE Jr. Asthma deaths during sports: report of a 7-year experience. *J Allergy Clin Immunol* 2004;113:264–7. [PubMed: 14767440]
29. Kim KC, Nassiri J, Brody JS. Mechanisms of airway goblet cell mucin release: studies with cultured tracheal surface epithelial cells. *Am J Respir Cell Mol Biol* 1989;1:137–43. [PubMed: 2695148]
30. Henderson WR Jr, Chiang GK, Tien YT, Chi EY. Reversal of allergen-induced airway remodeling by CysLT₁ receptor blockade. *Am J Respir Crit Care Med* 2006;173:718–28. [PubMed: 16387808]
31. Omori C, Tagari P, Freed AN. Eicosanoids modulate hyperpnea-induced bronchoconstriction in canine peripheral airways. *J Appl Physiol* 1996;81:1255–63. [PubMed: 8889761]
32. Lamb JP, Sparrow MP. Three-dimensional mapping of sensory innervation with substance p in porcine bronchial mucosa: comparison with human airways. *Am J Respir Crit Care Med* 2002;166:1269–81. [PubMed: 12403698]
33. Kuo HP, Rohde JA, Tokuyama K, Barnes PJ, Rogers DF. Capsaicin and sensory neuropeptide stimulation of goblet cell secretion in guinea-pig trachea. *J Physiol* 1990;431:629–41. [PubMed: 1712847]
34. Lai YL, Lee SP. Mediators in hyperpnea-induced bronchoconstriction of guinea pigs. *Naunyn-Schmiedeberg's Arch Pharmacol* 1999;360:597–602. [PubMed: 10598800]
35. Freed AN, McCulloch S, Meyers T, Suzuki R. Neurokinins modulate hyperventilation-induced bronchoconstriction in canine peripheral airways. *Am J Respir Crit Care Med* 2003;167:1102–8. [PubMed: 12574071]
36. Crimi N, Mastruzzo C, Pagano C, Lisitano N, Palermo F, Vancheri C. Montelukast protects against bradykinin-induced bronchospasm. *J Allergy Clin Immunol* 2005;115:870–2. [PubMed: 15806012]
37. Crimi N, Pagano C, Palermo F, Mastruzzo C, Prosperini G, Pistorio MP, et al. Inhibitory effect of a leukotriene receptor antagonist (montelukast) on neurokinin A-induced bronchoconstriction. *J Allergy Clin Immunol* 2003;111:833–9. [PubMed: 12704366]
38. Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, et al. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A* 2000;97:6155–60. [PubMed: 10823958]
39. Ellis JL, Undem BJ. Role of peptidoleukotrienes in capsaicin-sensitive sensory fibre-mediated responses in guinea-pig airways. *J Physiol* 1991;436:469–84. [PubMed: 2061842]
40. Forsythe P, McGarvey LP, Heaney LG, MacMahon J, Ennis M. Sensory neuropeptides induce histamine release from bronchoalveolar lavage cells in both nonasthmatic coughers and cough variant asthmatics. *Clin Exp Allergy* 2000;30:225–32. [PubMed: 10651775]
41. Barnes PJ. Neurogenic inflammation in the airways. *Respir Physiol* 2001;125:145–54. [PubMed: 11240158]
42. Naline E, Devillier P, Drapeau G, Toty L, Bakdach H, Regoli D, et al. Characterization of neurokinin effects and receptor selectivity in human isolated bronchi. *Am Rev Respir Dis* 1989;140:679–86. [PubMed: 2476956]
43. Fahy JV, Wong HH, Geppetti P, Reis JM, Harris SC, Maclean DB, et al. Effect of an NK1 receptor antagonist (CP-99,994) on hypertonic saline-induced bronchoconstriction and cough in male asthmatic subjects. *Am J Respir Crit Care Med* 1995;152:879–84. [PubMed: 7663799]
44. Ichinose M, Miura M, Yamauchi H, Kageyama N, Tomaki M, Oyake T, et al. A neurokinin 1-receptor antagonist improves exercise-induced airway narrowing in asthmatic patients. *Am J Respir Crit Care Med* 1996;153:936–41. [PubMed: 8630576]

FIG 1.

Gene expression of the gel-forming mucins MUC2, MUC5AC, and MUC5B in baseline induced sputum by quantitative polymerase chain reaction (A). The gel-forming mucin MUC6 was also measured but was not expressed. Gene expression of EGF ligands EGF, HB-EGF, amphiregulin (AREG), and TNF- α converting enzyme (TACE/ADAM17) in baseline induced sputum (B).

FIG 2.

Comparison of the expression of MUC5AC in induced sputum cells at baseline and on a separate day 30 minutes after exercise challenge in patients with asthma with EIB. The induced sputums were conducted a mean of 9.5 days apart. The expression of MUC5AC increased after exercise challenge (A) and was associated with the severity of EIB by regression analysis (B).

FIG 3.

Levels of secreted MUC5AC in induced sputum at baseline and 30 minutes after exercise challenge. Secreted MUC5AC was measured on a relative scale with the highest concentration on the standard curve set at a value of 1000 units.

FIG 4. Association between the levels of secreted MUC5AC in induced sputum and the levels of cysLTs (**A**) and 15S-HETE (**B**) after exercise challenge.

FIG 5.
Association between the levels of secreted MUC5AC in induced sputum and the levels of NKA after exercise challenge.

TABLE I

Characteristics of study participants

	Asthma with EIB (n = 22) [*]
Age (y) (range)	28.2 (14-55)
Gender (M/F)	11/11
Race	
Caucasian	90.9%
African American	4.6%
Asian	4.6%
Baseline	
FEV ₁ (% predicted)	84.8 ± 9.4
FVC (% predicted)	100.5 ± 10.9
FEV ₁ /FVC	0.72 ± 0.08
Postbronchodilator	
Δ FEV ₁ (%)	10.8 ± 6.1
Δ FVC (%)	2.5 ± 6.4
Postexercise	
Δ FEV ₁ (max)	28.4 ± 9.9
AUC ₃₀ [†] FEV ₁	664.3 ± 290.3

M, Male; F, female.

^{*} Values reported are mean ± standard deviation unless otherwise specified.

[†] Area under the curve (AUC) for the percent decrease in FEV₁ over 30 minutes after exercise.