Published in final edited form as:

Immunol Allergy Clin North Am. 2013 August ; 33(3): 313–vii. doi:10.1016/j.iac.2013.02.003.

Role of Cells and Mediators in Exercise-Induced Bronchoconstriction

Teal S. Hallstrand, MD, MPH^{a,*}, William A. Altemeier, MD^a, Moira L. Aitken, MD^a, and William R. Henderson Jr, MD^b

^aDivision of Pulmonary and Critical Care, Department of Medicine, University of Washington, 1959 NE Pacific Street, Box 356522, Seattle, WA 98195-6522, USA

^bDivision of Allergy and Infectious Diseases, Department of Medicine, UW Medicine at South Lake Union, University of Washington, 850 Republican Street, Seattle, WA 98109-4725, USA

Keywords

Asthma; Eicosanoid; Eosinophil; Exercise-induced bronchoconstriction; Leukotriene; Mast cell; Phospholipase; Prostaglandin

INTRODUCTION

The role of inflammatory mediators in the pathogenesis of exercise-induced bronchoconstriction (EIB) has become increasingly clear from studies conducted in human subjects with asthma over the last 15 years. These results indicate very clearly that mediators from mast cells and other airway leukocytes are released into the airways following exercise challenge in individuals who are susceptible to EIB. The evidence that the release of such mediators plays a causal role in the pathogenesis of EIB is strongest for the cysteinyl leukotrienes (CysLTs) C₄, D₄, and E₄, in part because of the availability of receptor antagonists and synthesis inhibitors that alter the leukotriene (LT) pathway. It is also apparent that several other mediators that may play important roles in the pathogenesis of EIB are released into the airways, but the precise roles of these mediators are inferred from animal studies and from the basic biological function of the mediators. Many unanswered questions remain, including why leukocytes become activated in the airways, how either evaporative water loss or the addition of a hyperosmolar solution to the airways initiates downstream cellular effects, and what the connection is between the epithelium and these events that leads to leukocyte activation.

ALTERATIONS IN THE AIRWAYS THAT LEAD TO EIB

As a prototypical feature of indirect airway hyperresponsiveness (AHR), EIB shares common features with other stimuli such as hypertonic aerosols and adenosine, which cause bronchoconstriction through the release of mediators.¹ EIB is only weakly related to structural alterations of the lung^{2–4} and airway smooth-muscle hyperresponsiveness measured by direct-acting agonists of smooth-muscle contraction such as methacholine.⁵ In one study of 27 asthmatic children, there was no relationship between the methacholine PC₂₀ and maximum decrease in forced expiratory volume in 1 second (FEV₁) after exercise

($r = -0.2$, $P = .40$). Another study of elite athletes found that 9 of 25 elite athletes with a positive eucapnic voluntary hyperpnea (EVH) challenge, a surrogate for exercise challenge, had a positive methacholine challenge, demonstrating the discordance between these 2 different features of AHR.⁶ Collectively, these studies indicate that EIB is pathophysiologically distinct from other features of asthma, but shares common features with other forms of indirect AHR. A clear understanding of the pathophysiology of EIB is important, as EIB at an early age is associated with the persistence of asthma later in life.⁷⁻⁹ There is also evidence that chronic lung disease early in life is a risk factor for the development or persistence of EIB later in life.¹⁰

As subjects with asthma can be characterized based on the presence or absence of EIB using a dry air exercise challenge test, several studies have made comparisons between asthmatics with and without EIB to better understand the basis for EIB. An inflammatory basis of EIB is suggested by an increase in the fraction of exhaled nitric oxide (F_{ENO}) among asthmatics who are susceptible to EIB,¹¹ especially in subjects with atopy.¹² Although differences in the concentration of inflammatory lipid mediators have not been identified in studies evaluating metabolites in the urine,¹³ the concentrations of inflammatory lipid mediators are increased in the airways of individuals with EIB.^{2,4,14} In particular, the concentration of CysLTs is increased in induced sputum of adults with EIB,⁴ and in exhaled breath condensate (EBC) of children with EIB.² In addition, the levels of 8-isoprostanes, nonenzymatic products of phospholipid oxidation, are increased in EBC of asthmatics with EIB, and correlate with the severity of EIB.¹⁴ There is also evidence of a reduction in the formation of protective lipid mediators in the airways such as lipoxin A4 in patients with EIB.¹⁵ Prostaglandin E₂ (PGE₂) is a key regulatory eicosanoid that inhibits EIB when administered by inhalation.¹⁶ The production of PGE₂ relative to CysLTs is reduced in patients with EIB.⁴ As the epithelium is a major source of PGE₂, it is notable that the number of epithelial cells shed into the induced sputum is greater in patients with EIB.⁴

The intensity of cellular inflammation in the airways may be an important factor in the susceptibility to EIB, as the formation of inflammatory eicosanoids such as CysLTs and PGD₂ is largely restricted to myeloid cells, especially mast cells that contain leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂) synthases and eosinophils that also contain LTC₄ synthase.¹⁷ Several studies have associated the degree of sputum eosinophilia with the severity of EIB,⁴ although sputum eosinophilia is not present in all patients with EIB.⁴ A recent study of the effect of inhaled corticosteroid (ICS) ciclesonide in steroid-naïve asthmatic patients with EIB found that the magnitude and onset of the suppression of EIB in response to high-dose, but not low-dose, ICS therapy was associated with the degree of sputum eosinophilia.¹⁸

In a genome-wide expression study of airway cells, the authors identified a unique molecular phenotype of EIB-positive asthmatics relative to asthmatic individuals without EIB, notable for the increased expression of mast cell, mucus, and epithelial repair genes.¹⁹ These results are consistent with other studies that found mast-cell involvement in EIB.²⁰ The authors found that the mast-cell genes tryptase and carboxypeptidase A3 (CPA3) were significantly increased in EIB-positive asthmatics, but the expression of chymase was unaltered¹⁹; these findings are particularly note-worthy, because the appearance of secretory granule proteases is regulated by the peripheral tissue, and most prior studies have found that the mucosal mast cells express tryptase, but not CPA3 or chymase.²¹ This intraepithelial mast-cell phenotype with high expression of tryptase and CPA3, but low expression of chymase, was recently described in the T-helper type 2 (Th2) high phenotype of asthma.^{22,23} Because this Th2 high phenotype is driven by interleukin (IL)-13,²⁴ it is interesting that a genetic study found an association between IL-13 gene polymorphisms and the severity of EIB, and with the treatment response to a CysLT₁ receptor antagonist among

these subjects with EIB.²⁵ In making comparisons between asthmatics with and without EIB, the authors also identified a selective increase in the levels of the complement component C3a in individuals with EIB.²⁶ Thus, patients who are susceptible to EIB have epithelial shedding, overproduction of inflammatory mediators such as CysLTs, relative underproduction of protective lipid mediators, and infiltration of the airways with eosinophils and mast cells (Fig. 1).

INFLAMMATORY MEDIATOR RELEASE DURING EIB

During periods of high ventilation, large volumes of air are equilibrated to the humidified body conditions of the lower airways over a short period of time, leading to the transfer of water, with resulting stress to the epithelium and cooling of the airways, largely as a result of water transfer.^{27–31} During tidal breathing at rest, little if any of the conditioning of the inspired air takes place beyond the upper airway and proximal trachea; however, conditioning of the inspired air takes place farther along the tracheobronchial tree as ventilation increases, forcing incompletely conditioned air to move deeply into the distal airways before it is brought to body conditions.³² This movement of water out of the airways triggers the release of mediators predominantly from airway leukocytes. The cellular mechanism leading to the activation of leukocytes, either directly through the movement of water or via a signal from the epithelium in response to this water movement, is not known in detail. There is strong evidence that leukocyte-derived eicosanoids including CysLTs and PGD₂ are released into the airways following an exercise challenge as assessed by induced sputum analysis.^{20,33} Similarly, in an analysis of EBC, the levels of CysLTs increased after exercise challenge, most notably in the EIB-positive group; furthermore, the change in CysLTs in EBC following challenge was correlated with the severity of EIB.³⁴ In nonasthmatic subjects, a gene-expression profiling study of peripheral blood following exercise challenge found increases in the expression of 5-lipoxygenase (5-LO) and 5-LO-activating protein (FLAP) in response to exercise challenge, as well as increased levels of LTs in the peripheral blood.³⁵

The formation of LTs and other eicosanoids is initiated by the release of unesterified arachidonic acid by the hydrolysis at the sn-2 position of membrane phospholipids by phospholipase A₂ (PLA₂) (Fig. 2). Arachidonic acid is transferred by FLAP to 5-LO, initiating the oxygenation of arachidonic acid to 5(*S*)-hydroperoxyeicosatetraenoic acid (5*S*-HpETE), followed by dehydration to the unstable epoxide leukotriene A₄ (LTA₄).³⁶ The critical enzyme in the formation of CysLTs from LTA₄ is the enzyme LTC₄ synthase (LTC₄S), part of a family of membrane-bound proteins involved in eicosanoid and glutathione metabolism including FLAP, microsomal glutathione *S*-transferases (MGSTs), and microsomal prostaglandin E synthase 1. LTC₄S conjugates glutathione with a high degree of substrate selectivity for LTA₄,^{37,38} leading to the generation of LTC₄ that is exported from the cell and rapidly metabolized to LTD₄ and then LTE₄.³⁹ The effects of LTD₄, including bronchoconstriction and airway inflammation, are mediated through the CysLT₁ receptor; however, the vast majority of CysLTs exist in the stable form of LTE₄ that does not interact with the CysLT₁ receptor.³⁹ Recent landmark studies have identified 2 different receptors for LTE₄ that participate in the development of allergic inflammation in animal models.⁴⁰ The incomplete effectiveness of CysLT₁ receptor antagonists may be explained in part by the presence of these additional receptors for LTE₄.

Because the expression of 5-LO is largely restricted to myeloid cells, the majority of LT synthesis occurs in leukocytes; however, arachidonic acid and intermediates such as LTA₄ are permeable across cell membranes, allowing for the transcellular metabolism of eicosanoids. Several studies have demonstrated that eicosanoid production in leukocytes is increased when the leukocyte is cocultured with a structural cell such as an epithelial cell.⁴¹

An important recent study demonstrated that 5-LO-deficient mice transplanted with immune cells deficient in LTC₄S were able to make normal quantities of CysLTs, demonstrating that 5-LO-containing immune cells transfer intermediates that restore LT synthetic capacity by transcellular metabolism.⁴²

The participation of epithelial cells in the release of mediators is suggested by several observations. First, the level of the epithelial-derived 15-lipoxygenase product 15*S*-hydroxyeicosatetraenoic acid (15*S*-HETE) is increased after exercise challenge, and is elevated in asthmatics compared with controls after exercise challenge.⁴³ The other mechanism involves the relative underproduction of epithelial-derived PGE₂ in patients with EIB. Following exercise challenge the level of PGE₂ declines in the airways of asthmatics with EIB,²⁰ whereas PGE₂ tends to increase in normal subjects.⁴³ This relative imbalance in epithelial-derived PGE₂ when compared with leukocyte-derived LTs supports the hypothesis that transcellular transport of epithelial-derived arachidonic acid or a regulator of arachidonic acid release contributes to LT synthesis in leukocytes, favoring bronchoconstriction in the period following exercise challenge (Fig. 3).⁴³ One explanation for these findings is that the epithelium serves to activate the production of inflammatory eicosanoids by leukocytes that are in close contact, and that there is shunting of epithelial-derived arachidonic acid away from the epithelial-derived PGE₂. In cell culture, inflammatory cells cocultured with epithelial cells have increased synthesis of leukocyte-derived eicosanoids.⁴⁴ Under the influence of IL-13, epithelial cells have reduced capacity for PGE₂ synthesis through a reduction in the synthetic enzymes cyclooxygenase-2 (COX-2) and PGE synthase 1.⁴⁵ The underproduction of PGE₂ could also directly alter the formation of CysLTs and PGD₂, as inhaled PGE₂ regulates the levels of these eicosanoids after segmental allergen challenge⁴⁶ and PGE₂ directly alters CysLT formation in cultured mast cells.⁴⁷

There is strong evidence that CysLTs play a causative role in the development of bronchoconstriction following exercise, through early proof-of-concept pharmacologic studies with CysLT₁ antagonists and 5-LO inhibitors. The CysLT₁ antagonist zafirlukast, given by inhalation to 9 patients with EIB 30 minutes before an exercise challenge, reduced the mean maximal percent decrease in FEV₁ to 14.5%, compared with 30.2% during placebo administration.⁴⁸ Oral zafirlukast was also shown to effectively inhibit EIB 2 hours after a single dose in a crossover study of 8 patients, resulting in a reduction in the mean maximal percent decrease in FEV₁ after challenge from 36.0% on placebo to 21.6% on zafirlukast.⁴⁹ A larger recent study showed that montelukast significantly reduced the severity of EIB at 2, 12, and 24 hours after a single dose, based on the maximum decrease in FEV₁ (10.8% montelukast vs 22.3% placebo at 2 hours, *P* .001) and area under the curve for the percentage decrease in FEV₁.⁵⁰ A second similarly designed study of 62 patients with EIB also showed similar efficacy at 2 hours after a single dose of montelukast compared with placebo (11.7% montelukast vs 17.5% placebo, *P* .001).⁵¹ In a similar manner, the 5-LO inhibitor zileuton administered 4 times daily for 2 days reduced the decrease in FEV₁ after challenge, from 28.1% on placebo to 15.6% on zileuton.⁵² These results clearly demonstrate a role for CysLTs in the pathogenesis of EIB but also indicate that the protection from EIB is incomplete, suggesting that mediators other than the CysLTs may play a significant role and that the loss of bronchoprotective mediators may be important. A recent study found that an ICS added to a CysLT₁ antagonist had improved efficacy over treatment with either an ICS or CysLT₁ antagonist alone.⁵³

CELLULAR ACTIVATION DURING EIB

Mast cells and eosinophils are strongly implicated as the cellular sources of CysLTs and other eicosanoids such as PGD₂ in EIB. The eosinophil product eosinophilic cationic protein

(ECP) is released into the airways following challenge, and the amount of ECP release varies with the severity of the EIB under different experimental conditions.³³ Following exercise challenge, histamine and the mast-cell protease tryptase are released into the airways, demonstrating mast-cell degranulation (Fig. 4).²⁰ Using the urinary levels of 9α , 11β -PGF₂ as a marker of PGD₂ metabolism and mast-cell activation in response to EVH, the release of PGD₂ can be inhibited by pretreatment with a cromone or a high dose of inhaled steroid, suggesting that PGD₂ release by mast cells may play an important role in the development of bronchoconstriction after exercise challenge.^{13,54} Using mannitol challenge as a surrogate for exercise, pharmacologic inhibitors indicate that histamine is responsible for bronchoconstriction early after challenge while the release of CysLTs is responsible for sustained bronchoconstriction.⁵⁵

CONTRIBUTION OF SENSORY NERVES

Sensory nerves release neurokinins when activated through a process called retrograde axonal transmission, leading to bronchoconstriction and mucus release. There is evidence in animal models that the production of eicosanoids such as CysLTs mediate bronchoconstriction, at least in part through the activation of airway sensory nerves. Although sensory nerves may be activated directly by osmotic stimuli, several eicosanoids can either directly activate or alter the activation threshold of sensory nerves.⁵⁶ In a dog model, a combination neurokinin-1 and neurokinin-2 receptor antagonist inhibited hyperpnea-induced bronchoconstriction (HIB) and the generation of LTs that are known in this model to cause HIB.⁵⁷ In a guinea pig model of HIB, either a 5-LO inhibitor or a CysLT₁ antagonist inhibited HIB and the release of neurokinins, whereas a neurokinin-2 receptor antagonist inhibited HIB but not the release of leukotrienes, suggesting that leukotrienes cause bronchoconstriction via sensory nerves during HIB.⁵⁸ In humans, the effects of neurokinin-1 antagonists have been modest^{59,60}; however, this lack of efficacy may be due to the predominance of neurokinin A, which binds predominantly to the neurokinin-2 receptor.⁶¹ As goblet-cell mucin release is initiated via neurokinin release, it is notable that mucin 5AC (MUC5AC), the predominant gel-forming mucin of goblet cells, is released into the airways during EIB in humans (Fig. 5) and is associated with the levels of CysLTs in the airways.⁶² Furthermore, the levels of neurokinin A and CysLTs in these individuals after exercise challenge are correlated, suggesting that CysLTs mediate the activation of sensory nerves and mucus release during EIB in humans.⁶²

LATE PHASE RESPONSE

Despite the very compelling evidence that exercise challenge acutely causes the release of inflammatory mediators into the airways, several studies failed to demonstrate a cellular influx into the airways or an increase in direct AHR following an exercise challenge.^{63–66} A physiologic late-phase response has also been difficult to demonstrate in many studies, or has been attributed to factors such as the inevitable fluctuation in airway tone among patients with asthma.^{67–69} Despite this controversy, some studies indicate that a portion of patients have a second wave of airflow obstruction consistent with a late-phase response to exercise challenge,^{70,71} and there is clearly a late-phase response in a dog model of HIB.⁷² The late-phase response to exercise challenge may also be inhibited by treatment with the CysLT₁ receptor antagonist montelukast.⁷⁰ Recent provocative results using EBC demonstrate an increase in high-sensitivity C-reactive protein only in asthmatics with EIB following exercise challenge.⁷³ In addition, the F_{ENO}, serum ECP, and AHR to inhaled histamine were all increased following exercise challenge in asthmatics with EIB.⁷³ Further, RANTES and eotaxin in EBC were increased in asthmatics relative to controls, and the levels of RANTES and eotaxin were increased in EBC after exercise challenge in the group with EIB but not in asthmatics without EIB.^{74,75} These data indicate that inflammatory

mediator release with exercise challenge may recruit leukocytes to the airways, but the magnitude of such leukocyte recruitment is small or counterregulated by anti-inflammatory pathways.

REGULATION OF EICOSANOID PRODUCTION AND LEUKOCYTE ACTIVATION BY THE EPITHELIUM

A unifying concept regarding the pathogenesis of EIB is that the epithelium may regulate the response to water loss experienced during exercise challenge. One possibility is that a regulator of eicosanoid formation is released by the airway epithelium in response to water loss. Water is transferred out of the airways during exercise, leading to the movement of water by the semipermeable and osmotically sensitive epithelium that rapidly corrects alterations in airway surface liquid (ASL) osmolarity (Fig. 6).⁷⁶ A similar epithelial response with the transfer of water should occur in response to exogenous osmolar stimuli such as mannitol and hypertonic saline. In response to water loss in the luminal surface, epithelial cell height reduces as the cells transfer water to the surface.^{77,78} Because of the ability of the epithelium to respond to movement of water, there is little evidence that any osmotic gradient exists more than transiently in the ASL.⁷⁹ However, evaporative water loss from epithelial cells initiates the release of cellular adenosine triphosphate and adenosine, leading to the activation of chloride channels and increasing intracellular calcium.⁷⁶ This stress to the epithelium during exercise may explain the increased levels of adenosine in EBC following exercise challenge that correlate with the severity of EIB.⁸⁰ It is also known that osmotic stimuli can directly activate inflammatory cells such as mast cells⁸¹; however, hyperosmolarity per se exists only transiently in the airways, and likely serves as a stimulus primarily to the epithelium. The connection between epithelial stress induced by water loss and the activation of leukocytes in the airways needs to be understood more completely.

The first rate-limiting step in eicosanoid formation is the release of arachidonic acid mediated by PLA₂. It is well known that cytosolic PLA₂α (cPLA₂α) functions as a major regulator of efficient eicosanoid synthesis.⁸² More recently, a family of secreted PLA₂s (sPLA₂) have been described that can also serve as regulators of eicosanoid synthesis and may preferentially direct eicosanoid production toward LT synthesis.³⁹ It has been known for some time that sPLA₂ activity in the airways increases following allergen challenge in the upper and lower airways.^{83–85} Of the mammalian sPLA₂s, groups V and X have generated the most interest because of their capacity to initiate cellular eicosanoid synthesis,⁸⁶ particularly sPLA₂ group X (sPLA₂-X), because it is the most potent of the sPLA₂s at releasing arachidonic acid from membrane phospholipids. In murine models of asthma, genetic deficiency of either sPLA₂-V or sPLA₂-X attenuates the development of allergen-induced inflammation, mucus release, and AHR,^{87,88} as does the pharmacologic inhibition of human sPLA₂-X expressed in a transgenic mouse model.⁸⁹ The authors have found that sPLA₂-X levels are increased in the bronchoalveolar lavage fluid of patients with asthma and that sPLA₂-X is predominantly expressed in the airway epithelium.⁹⁰ In addition, using semiquantitative techniques they have found that sPLA₂-X protein in induced sputum supernatant and epithelial cells immunostaining for sPLA₂-X in induced sputum increases following exercise challenge, suggesting that activation or release of sPLA₂-X may be involved in the generation of eicosanoids following exercise challenge.⁴³

The release of sPLA₂-X or other similar enzymes into the airways after exercise challenge could play an important role in the pathogenesis of EIB through the initiation of mediator formation in leukocytes, the degradation of surfactant phospholipids, and the generation of lysophospholipids. The authors examined the ability of sPLA₂-X to efficiently activate CysLT formation by human eosinophils.⁹¹ Recombinant human sPLA₂-X initiates arachidonic acid release and CysLT synthesis through a mechanism that is dependent on the

enzymatic activity of sPLA₂-X, but could also be replicated by lysophospholipids released by eosinophils in response to sPLA₂-X. Of interest, the full mechanism of sPLA₂-X-mediated activation of eosinophils involves the activation of the mitogen-activated kinase cascade and the activation of cPLA₂α; however, CysLT formation is amplified by sPLA₂-X, probably as an additional source of arachidonic acid.

Another important finding is the increased expression of transglutaminase 2 (TGM2) in the airways of patients with EIB relative to asthmatics without EIB.¹⁹ It is notable that the TGM2 gene is located on chromosome 20q11.2-12 near a cluster of genes related to epithelial barrier function, in close proximity to a region linked to both atopic dermatitis and asthma.⁹² Using an in vitro assay of sPLA₂ activity, the authors found that recombinant human TGM2 enzymatically modifies sPLA₂-X, leading to a substantial increase in the sPLA₂ activity of the enzyme, suggesting that one of the mechanisms of TGM2 action in asthma is regulation of eicosanoid and lysophospholipid synthesis. The potential importance of this finding was highlighted in mouse models showing that TGM2 is induced in the airways of mice sensitized and challenged with ovalbumin in the presence of adjuvant,⁹³ as well as in mouse models of PMA-induced atopic dermatitis and immunoglobulin E (IgE)-dependent passive cutaneous anaphylaxis.⁹⁴ In one study a peptide that inhibits both TGM2 and PLA₂ reduced allergen-induced airway inflammation and eicosanoid formation, but the specific role of TGM2 remains to be fully elucidated.⁹³ A chemical inhibitor of TGM2 partially inhibited PMA-induced dermatitis and IgE-dependent cutaneous anaphylaxis.⁹⁴ Collectively these studies suggest that the epithelium can serve as an important regulator of eicosanoid formation, and that the release of sPLA₂-X from the airway epithelium could regulate mediator formation in EIB.

SUMMARY

Exercise challenge leads to a distinct syndrome of bronchoconstriction that occurs in a susceptible group of subjects who have epithelial shedding, overproduction of inflammatory eicosanoids, and mast-cell and eosinophil infiltration of the airways. Exercise challenge causes water loss from the airways, leading to the movement of water from the epithelium to correct the resultant transient shift in osmolarity. Although the precise mechanism by which water loss leads to the activation of leukocytes is not known, it is clear that exercise challenge initiates the release of inflammatory mediators from leukocytes such as mast cells and eosinophils. In the case of the release of CysLTs, it is clear that the release of CysLTs acts via the CysLT₁ receptor to initiate bronchoconstriction, based on studies in humans using pharmacologic inhibitors. Other mediators such as 15*S*-HETE and PGD₂ are released from the airway cells, but the specific role of these mediators in the development of bronchoconstriction is less certain. Based predominantly on animal and in vitro studies, sensory nerves seem to play an important role in either transmitting the effects of eicosanoids that are released in the airways or as direct sensors of changes in airway osmolarity, a process that is potentiated by elevated levels of eicosanoids. A possible explanation for the cellular events in the airways is that the airway epithelium, acting as the primary sensor of water loss, releases a product that serves to activate leukocytes that are in close contact with the epithelium, leading to the release of leukocyte-derived eicosanoids and activation of sensory nerves. One such product is sPLA₂-X, which is avidly expressed in the airway epithelium, increased in the airway fluid of asthmatics, and serves to activate airway cells such as eosinophils to generate CysLTs. The regulation of these cellular events requires further study so that targeted therapies may be developed to modulate this important aspect of asthma.

Acknowledgments

Funding: Supported by National Institutes of Health grant HL089215.

T.S. Hallstrand has received research grants from the NIH, American Lung Association, has served as a consultant for Amgen and TEVA pharmaceuticals, and has received lecture fees from Merck & Co. W.R. Henderson has served on advisory boards for Gilead Sciences, has received lecture fees from Merck & Co., and has received industry-sponsored grants from Genentech and Gilead Sciences.

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KEY POINTS

- Patients who are susceptible to exercise-induced bronchoconstriction have epithelial shedding, infiltration of the airways with mast cells and eosinophils, and increased production of inflammatory mediators such as leukotrienes.
- During exercise and hyperpnea the inspired air is equilibrated to the conditions of the lower airways, resulting in the transfer of water out of the airways.
- Following exercise challenge, mediators such as cysteinyl leukotrienes and prostaglandin D₂ are released into the airways from mast cells and eosinophils.
- Sensory nerves may mediate the effects of cysteinyl leukotrienes and other lipid mediators, leading to smooth-muscle contraction and mucus release.
- The epithelium may serve as a regulator of leukocyte activation in response to water loss or osmotic stress, but the mechanism remains incompletely understood.

Fig. 1.

Disease model of exercise-induced bronchoconstriction (EIB) pathogenesis. Studies examining differences between asthmatics with EIB to those without EIB have identified an increased concentration of shed epithelial cells, and infiltration of the airways with eosinophils and mast cells. There is an alteration in the balance of lipid mediators notable for an increase in cysteinyl leukotrienes (CysLTs), CysLT/prostaglandin E₂ (PGE₂) ratio, and 8-isoprostanes, and a reduction in lipoxin A4. eNO, exhaled nitric oxide. (*Adapted from Hallstrand TS, Henderson WR Jr. Role of leukotrienes in exercise-induced bronchoconstriction. Curr Allergy Asthma Rep 2009;9:18–25.*)

Fig. 2.

Eicosanoid formation from arachidonic acid via the 5- and 15-lipoxygenase and cyclooxygenase pathways. COX, cyclooxygenase; CysLT, cysteinyl leukotriene; FLAP, 5-lipoxygenase-activating protein; HETE, hydroxyeicosatetraenoic acid; LO, lipoxygenase; PG, prostaglandin; PLA₂, phospholipase A₂; TX, thromboxane.

Fig. 3.

Ratio of CysLTs to PGE₂ in induced sputum following exercise challenge. The levels of CysLTs and PGE₂ were measured in induced sputum in a group of asthmatics with EIB (A) and in a nonasthmatic control group (B) at baseline and after exercise challenge. The results demonstrate opposing effects of an increase in the CysLT/PGE₂ ratio after challenge in the asthma group, whereas the ratio decreased after challenge in the control group. (Data from Hallstrand TS, Chi EY, Singer AG, et al. Secreted phospholipase A2 group X over-expression in asthma and bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2007;176:1072–8.)

Fig. 4.

Mast-cell degranulation following exercise challenge in asthmatics with EIB. The levels of histamine (*A*) and tryptase (*B*) were measured in induced sputum supernatant at baseline and on a separate day 30 minutes after exercise challenge. Significant increases in both histamine and tryptase were observed following exercise challenge in subjects with EIB. (Data from Hallstrand TS, Moody MW, Wurfel MM, et al. Inflammatory basis of exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2005;172:679–86.)

Fig. 5.

Goblet cell mucin release following exercise challenge in asthmatics with EIB. The levels of the gel-forming mucin MUC5AC were measured before and after exercise challenge in induced sputum supernatant in subjects with EIB. The induced sputum samples were dialyzed to remove the dithiothreitol (DTT) before analysis. A significant increase in MUC5AC was observed following exercise challenge. (*Data from Hallstrand TS, Debley JS, Farin FM, et al. Role of MUC5AC in the pathogenesis of exercise-induced bronchoconstriction. J Allergy Clin Immunol 2007;119:1092–8.*)

Fig. 6.

Basic overview of the epithelial response to water loss from the airway surface liquid. As ventilation increases, water is lost from the airway surface liquid, resulting in transient hyperosmolarity and the passive movement of water from airway epithelial cells (*arrows*) to restore the osmolarity of the airway surface liquid. The movement of water causes epithelial cells to shrink and initiates cellular signaling events, including an increase in intracellular calcium (Ca^{2+}). The addition of osmotically active substances such as mannitol and hypertonic saline similarly cause water movement to restore the surface osmolarity.