Epithelial regulation of eicosanoid production in asthma

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Alterations in the airway epithelium have been associated with the development of asthma in elite athletes and in subjects that are susceptible to exercise-induced bronchoconstriction (EIB). The syndrome of EIB refers to acute airflow obstruction that is triggered by a period of physical exertion. Asthmatics who are susceptible to EIB have increased levels of cysteinyl leukotrienes (CysLTs, i.e., LTs C4, D4, and E4) in induced sputum and exhaled breath condensate, and greater shedding of epithelial cells into the airway lumen. Exercise challenge in individuals susceptible to this disorder initiates a sustained increase in CysLTs in the airways, and secreted mucin release and smooth muscle constriction, which may be mediated in part through activation of sensory nerves. We have identified a secreted phospholipase A2 (sPLA2) with increased levels in the airways of patients with EIB called sPLA2 group X (sPLA2-X). We have found that sPLA2-X is strongly expressed in the airway epithelium in asthma. Further, we discovered that transglutaminase 2 (TGM2) is expressed at increased levels in asthma and serves as a regulator of sPLA2-X. Finally, we demonstrated that sPLA2-X acts on target cells such as eosinophils to initiate cellular eicosanoid synthesis. Collectively, these studies identify a novel mechanism linking the airway epithelium to the production of inflammatory eicosanoids by leukocytes. © 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Asthma can be viewed as a group of related phenotypes with significant heterogeneity in both the underlying genetic and environmental determinants as well as in the clinical manifestations of the disease [1]. A prominent manifestation of asthma is exercise-induced bronchoconstriction (EIB), a syndrome where a brief period of exercise or increase in ventilation triggers airflow obstruction that lasts 30–90 min in the absence of treatment [2]. We have conducted a number of studies that examine the immunological basis of EIB, and mediator release in the airways following exercise challenge. Here we review the underlying immunopathology that leads to EIB, and the nature of mediator release in the airways following exercise challenge. These studies serve as the foundation of our work examining the regulation of mediator formation in the airways, particularly the identification of sPLA2-X as an important regulator of eicosanoid formation [3–6], the discovery that transglutaminase 2 (TGM2) is expressed at increased levels in asthma and serves as a regulator of sPLA2-X. Finally, we demonstrated that sPLA2-X acts on target cells such as eosinophils to initiate cellular eicosanoid synthesis [7]. Collectively, these studies identify a novel mechanism linking the airway epithelium to the production of inflammatory eicosanoids by leukocytes in the airways.

2. Clinical implications

The importance of EIB within the spectrum of asthma is due to the impact of the exercise-related symptoms and airflow obstruction. Exercise-related asthma symptoms are associated with reduced health-related quality of life in children [9], and exercise challenge can serve as a stimulus for severe bronchoconstriction.
3. Immunopathology of asthma with Exercise-induced Bronchoconstriction (EIB)

Cross sectional studies in adults suggest that EIB is a discrete phenotype with distinct pathophysiology that is most strongly related to other aspects of indirect AHR [15]. Whether or not this phenotype is a durable clinical phenotype awaits further longitudinal epidemiological studies. A modest size cross-sectional study established a prevalence of EIB of 46% out of 164 asthmatic children who were not using any daily controller therapy, and found that the prevalence of EIB was increased in the children whose asthma was more severe [16]. The severity of EIB is generally not associated with the baseline forced expiratory volume in 1 s (FEV1) [12,16,17], and is only weakly associated with the degree of direct AHR [18,19].

An inflammatory basis of EIB is suggested by an increase in the fraction of exhaled nitric oxide (FENO) among asthmatics who are susceptible to EIB [20], especially in subjects with atopy [21]. In a comparison that we conducted between two groups of asthmatics, one with EIB and the other without EIB, we found that the concentration and number of columnar epithelial cells in induced sputum was much higher in asthmatics with EIB, suggesting that the epithelium is disrupted, and epithelial cells are shed into the airway lumen in this disorder [2]. We found that the concentration of cysteiny luteokienes (CysLTs, LTs C4, D4 and E4) are increased in induced sputum of adults with EIB [2], and another group found that the levels of CysLTs are increase in exhaled breath condensate of children with EIB [17]. Eicosanoids such as LTs and prostanlandins (PGs) are formed from arachidonic acid (AA) that is released by the hydrolysis of the sn-2 position of membrane phospholipids by a family of phospholipase A2 (PLA2) enzymes. Although AA and many of the products of AA readily move across the cell membrane, the formation of inflammatory eicosanoids such as CysLTs and PGD2 is largely restricted to myeloid cells, especially mast cells and eosinophils that contain LT4C synthase and mast cells that contain PGD2 synthase [22]. The connection between epithelial shedding and increased production of inflammatory lipid mediators has led us to consider mechanisms involving the epithelial regulation of leukocyte mediator production and function. One aspect of this relationship between the epithelium and leukocytes is that the epithelium that inhibits EIB [23] is consistently decreased in relation to the generation of CysLTs in subjects with EIB [2].

We found that the number of eosinophils was increased overall in subjects with EIB, but sputum eosinophilia per se does not appear to be required for EIB [2]. In line with these observations the magnitude and onset of the suppression of EIB in response to high dose but not low dose inhaled corticosteroid (ICS) therapy was associated with the degree of sputum eosinophilia [24]. Subjects with EIB who did not have sputum eosinophilia were less likely to have an improvement in EIB during ICS therapy compared to those with evidence of sputum eosinophilia [24]. We also found in a genome-wide expression study of airway cells of patients with EIB relative to patients with asthma that did not have EIB, that the expression of the mast cell genes tryptase and carboxypeptidase A3 (CPA3) were significantly increased in the EIB positive group [7]. These data are consistent with the recent findings of a unique intraepithelial mast cell phenotype in asthma notable for the high expression of tryptase and CPA3, but low expression of chymase that was described particularly in the Th2 high molecular phenotype [25,26]. Collectively these studies indicate that patients with EIB represent a group of subjects with prominent cellular inflammation and epithelial shedding into the airway lumen in association with increased production of inflammatory eicosanoids.

4. Inflammatory mediator release in the airways during EIB

Studies conducted in our lab and others indicate that exercise challenge initiates the release of inflammatory mediators into the airways in asthmatics with EIB, but the precise mechanism that initiates these events remains an area of controversy [27]. Under most conditions, heat and water are transferred out of the airways during exercise as the inspired air is equilibrated to the temperature and humidity of the lower airways. The amount of water transferred out of the airways during exercise is strongly associated with the severity of bronchoconstriction following exercise challenge. It is likely that water transfer from the airways during exercise serves as a stimulus to the epithelium and leukocytes residing within the epithelium to initiate the release of mediators. Studies from our laboratory and others indicate that there is a sustained increase in CysLTs and other bronchoconstrictive eicosanoids such as PGD2 in the airways following exercise challenge to induce EIB [28,29]. The levels of CysLTs are elevated at 30 min, 1 h, and 6 h after exercise challenge in asthmatics with EIB [28,29]. It is clear from pharmacological inhibitor studies blocking either 5-lipoxygenase (5-LO) or the CysLT1 receptor that CysLTs, especially LTD4 plays a pathological role in this disorder [28,30–33]. However, the inhibition of EIB by CysLT inhibitors alone is incomplete, implicating other bronchoconstrictive eicosanoids and/or the reduction in bronchoprotective mediators such as PGE2 in the pathogenesis of EIB. Fish oil supplementation, high in n-3 polyunsaturated fatty acids (PUFA) that inhibit synthesis of 2-series PGs and the 4-series LTs, inhibits EIB and the increase in both CysLTs and PGD2 in the airways [34], and the mast cell product 9x,11x-PGF2 alters the urine following exercise challenge [35]. A major finding of these studies is that the epithelium itself may play a key role in the regulation and production of inflammatory mediators. Following exercise challenge the level of PGE2 declines in the airways of asthmatics with EIB [28], altering the balance of bronchoconstrictive to bronchoprotective mediators favoring bronchoconstriction in the period following exercise challenge [3]. A unifying explanation for these findings is that the epithelium regulates the production of inflammatory eicosanoids by leukocytes that are in close contact, and that there is shunting of epithelial-derived AA away from the epithelium and toward the production of inflammatory eicosanoids by adjacent leukocytes. Inflammatory cells co-cultured with epithelial cells in vitro have increased synthesis of leukocyte-derived eicosanoids [36]. Under the influence of interleukin-13 (IL-13) in vitro, the epithelium has reduced capacity for PGE2 synthesis through a reduction in the synthetic enzymes cyclooxygenase-2 (COX-2) and PGE synthase 1 [37]. The epithelium itself may also serve directly as an important source of inflammatory mediators such as the eicosanoid 15S-Hydroxyeicosatetraenoic Acid (15S HETE) that is increased in the airways of patients with EIB after exercise challenge [3]. Studies in asthma have found that the key enzyme in the 15S HETE synthetic pathway, 15-Lipoxygenase-1, has increased expression in the airway epithelium of patients with asthma [38,39]. These findings suggest
that alterations in the airway epithelium may serve to regulate the production of inflammatory eicosanoids.

Mast cells and eosinophils are strongly implicated as the cellular sources of CysLTs and other eicosanoids in EIB. The eosinophil product eosinophil 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 [29]. Following exercise challenge, histamine and the mast cell protease tryptase are released into the airways, and inhibition of EIB with a CysLT1 receptor inhibitor along with an antihistamine reduced the amount of histamine released after exercise challenge [28]. In an analogous situation using manit challenge, pharmacological inhibitors indicate that histamine is responsible for bronchoconstriction early after challenge, while the release of CysLTs is responsible for sustained bronchoconstriction [40].

5. Sensory nerve involvement in EIB

The production of eicosanoids such as CysLTs in the airway may initiate bronchoconstriction in part through the activation of sensory airway nerves. Sensory nerves release neurokinins when activated through a process called retrograde axonal transmission leading to bronchoconstriction and mucus release. Sensory nerves may be activated directly by osmotic stimuli, but several eicosanoids can significantly alter the activation threshold of these nerves. In a guinea pig model of hyperpnea-induced bronchoconstriction (HIB), either a 5-LO inhibitor or a CysLT1 antagonist inhibited HIB and the release of neurokinins, while a neurokinin 2 receptor antagonist inhibited HIB, but not the release of leukotrienes, suggesting that leukotrienes are involved in the release of neurokinins that cause bronchoconstriction [41]. Similarly in a dog model, a combination neurokinin 1 and 2 receptor antagonist inhibited HIB and the generation of LTs that are known in this model to cause HIB [42]. We demonstrated that mucin 5AC (MUC5AC), the predominant gel-forming mucin of goblet cells is released into the airways during EIB and is associated with the levels of CysLTs in the airways [43]. Further, the levels of neurokinin A and CysLTs in these individuals post-exercise challenge are correlated, suggesting that CysLTs mediate the activation of sensory nerves and mucus release during EIB in humans [43].

6. Identification of secreted PLA2 group X (sPLA2-X) in the airway epithelium as a potential regulator of eicosanoid production

The first rate-limiting step in the formation of the CysLTs and other eicosanoids is the release of arachidonic acid (AA) from membrane phospholipids that is regulated by the PLA2 enzymes. It is clear from many studies that cytosolic PLA2 (cPLA2) has a major function in efficient eicosanoid synthesis, evidenced by the marked reduction in eicosanoid production when the gene is knocked out in a murine model of asthma [44]. However, in the presence of cPLA2, several secreted PLA2s (sPLA2s) have been shown to significantly increase AA release over cPLA2 alone, and may preferentially direct eicosanoid production toward LT synthesis [45]. Although the identities of specific sPLA2s were not characterized, increased sPLA2 activity was identified in nasal lavage fluid and in bronchoalveolar lavage (BAL) fluid following allergen challenge [46–48]. To determine the identities of the sPLA2s in human airways, we examined induced sputum samples from asthmatics with EIB as well as a non-asthmatic control group and found that sPLA2 groups X and XIIA predominated at the level of gene expression [3]. Immunocytochemistry indicated that groups X and XIIA are primarily present in columnar epithelial cells and bronchial macrophages. Of the mammalian sPLA2s, groups V and X have generated the most interest because of their capacity to initiate cellular eicosanoid synthesis [49], particularly sPLA2 group X (sPLA2-X) since it is the most potent of the sPLA2s at releasing AA from membrane phospholipids. Because sPLA2-X is able to hydrolyze phosphatidylcholine-rich vesicles at a rate comparable with its action on anionic phospholipids, sPLA2-X releases AA when added exogenously to the phosphatidylcholine-rich extracellular plasma membrane of mammalian cells. 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 [6,50], as does inhibition of human sPLA2-X expressed in a transgenic mouse model [5]. In our initial study we found that, following exercise challenge in asthmatics with EIB, there were increases in sPLA2-X protein in induced sputum supernatant and the percentage of epithelial cells immunostaining for sPLA2-X, suggesting that activation or release of sPLA2-X may be involved in the generation of eicosanoids following exercise challenge [3]. In subsequent work to better understand the identities of the sPLA2s in human airways, we have found that sPLA2-X and sPLA2-IIA are the predominant sPLA2s in human BAL fluid both in subjects with and without asthma [4]. In the airway epithelium, the expression of sPLA2-X predominated (Fig. 1), while both sPLA2-X and sPLA2-IIA were expressed in BAL cells [4]. The levels of sPLA2-X in BAL fluid were increased in asthma, particularly in severe asthma and correlated with lung function and eicosanoid formation in the airways. In contrast, although sPLA2-IIA was elevated in asthma, it was not associated with lung function, cellular inflammation or eicosanoid levels [4]. Taken together, these results suggest a prominent role of sPLA2-X in asthma as a regulator of cellular inflammation and eicosanoid formation. Studies are currently underway to better understand the distribution of sPLA2-X expression within the epithelium of patients with asthma and non-asthmatic subjects. It is notable that in the murine model, the expression of sPLA2-X co-localizes to cells expressing MUC5AC suggesting prominent expression in secretory cells such as goblet cells [6].

7. Transglutaminase 2 (TGM2) is increased in the airways of patients with asthma and regulates the activity of sPLA2-X

In our comparison of genes expressed in airway cells, we found that the expression of TGM2 is increased in asthmatics with EIB relative to asthmatics without EIB, and that TGM2 is markedly increased in either asthma group relative to non-asthmatic controls [7]. Immunostaining for TGM2 in endobronchial biopsies from patients with asthma demonstrated TGM2 throughout the airway epithelium. In addition, primary epithelial cells proliferating in culture contain high amounts of TGM2. Although TGM2 has been implicated in a number of inflammatory diseases, our study was the first to clearly implicate TGM2 in asthma. Of interest is that TGM2 is upregulated by retinoic acid in transformed airway epithelial cells [51]. 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 [52]. Two of the other differentially expressed genes in our study, secretory leukocyte peptidase inhibitor (SLPI) and cystatin 1 (CST1), are also located in this region of chromosome 20. TGM2 is a calcium-dependent enzyme that modifies protein structure through the transfer of an acyl group from glutamine to lysine or free amines resulting in a new inter- or intra-molecular amidic cross-link [53]. TGM2 is also known to activate the transcription factor NFkB, which induces expression of pro-inflammatory cytokines [54]. Using an in vitro assay of PLA2 activity, we found that recombinant human TGM2 enzymatically modifies sPLA2-X leading to a substantial increase in the PLA2 activity of the enzyme, suggesting that one of the mechanisms of TGM2 action in asthma
is regulation of eicosanoid and lysophospholipid synthesis (Fig. 2). In a prior investigation, dual inhibitors of TGM2 and sPLA2 reduced ocular inflammation in a rabbit model of allergen-induced conjunctivitis [53]. It is now clear from more recent animal models that TGM2 is induced in the airways of mice sensitized and challenged with ovalbumin in the presence of adjuvant [55], as well as in mouse models of PMA-induced atopic dermatitis and IgE-dependent passive cutaneous anaphylaxis [56]. 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 [55]. In the studies of TGM2 in cutaneous anaphylaxis and atopic dermatitis, a chemical inhibitor of TGM2 partially inhibited PMA-induced dermatitis and IgE-dependent cutaneous anaphylaxis [55].

8. Secreted PLA2 group X (sPLA2-X) initiates eicosanoid production by human eosinophils

Eosinophils have been implicated as a significant source of CysLTs involved in the pathogenesis of EIB based on increased levels of eosinophils in association with both high levels of CysLTs [2] and the severity of EIB [24] as well as evidence of activation of eosinophils in the airways following exercise challenge [29]. The critical enzyme in CysLT formation, LTC4 synthase, is predominantly present in mast cells and eosinophils in the airways in subjects with asthma [22], and eosinophils are the predominant source of LTC4 synthase in aspirin exacerbated respiratory disease (AERD) [57]. Following allergen challenge, the amount of CysLT formation in the airways is associated with the eosinophil count, further implicating eosinophils as a major source of CysLT production [58].

Based on the identification of sPLA2-X in the airways of patients with EIB and the evidence that eosinophils are a major source of CysLTs, we examined the ability of sPLA2-X to efficiently activate CysLT formation by human eosinophils [8]. It is well known that group IVA cytosolic PLA2 (i.e. cPLA2a) plays a major role in endogenous CysLT synthesis in myeloid cells such as eosinophils [59,60]; however, it is reported that sPLA2 group V (sPLA2-V) initiates CysLT synthesis by human eosinophils in the absence of sPLA2-V activation [61,62]. Although sPLA2-V and sPLA2-X both have high capacity to initiate cellular eicosanoid synthesis
sPLA2-V has been difficult to identify in the airways of patients with asthma [4] or EIB [3]. To examine the role of sPLA2-X in eosinophil CysLT synthesis, we used recombinant human sPLA2-X to activate human eosinophils isolated from donors with a physician diagnosis of asthma and/or allergy. Exogenous sPLA2-X rapidly caused release of a large portion of labeled AA and CysLT synthesis that was related to the amount of sPLA2-X added exogenously to eosinophils. A specific, active site-directed inhibitor of sPLA2-X inhibited both CysLT synthesis and AA release indicating that sPLA2-X was responsible for the AA release and CysLT synthesis. In addition to AA release, sPLA2-X caused marked lysophospholipid release from eosinophils including lysophosphatidylcholine (PC), phosphatidylglycerol (PG), and plasmalogen PC and PE species [64], were released by the addition of sPLA2-X. Although it is clear that sPLA2-X serves as a major source of AA and lysophospholipids, the mechanism of CysLT formation is more complex. We found that selective inhibitors of cPLA2 suppressed CysLT formation mediated by sPLA2-X suggesting that sPLA2-X or a product of sPLA2-X activates cPLA2. Activation of cPLA2 involves an intracellular calcium flux and phosphorylation of a serine residue by MAP kinases. Treatment with sPLA2-X initiated Ser195-phosphorylation of cPLA2 and an intracellular Ca2+ flux in eosinophils, as well as translocation of cPLA2 and 5-LO to focal locations in the cytoplasm and in the perinuclear space. CysLT formation in response to sPLA2-X was attenuated by pharmacological inhibition of p38 and JNK MAP kinases; further, lysophosphatidylcholine induced cPLA2 was strongly expressed in the airway epithelium and initiates CysLT synthesis mediated by exogenous sPLA2-X. Finally, we demonstrated that sPLA2-X acts on target cells such as eosinophils to initiate cellular eicosanoid synthesis. Collectively, these studies identify a novel mechanism linking the airway epithelium to the production of inflammatory eicosanoids by leukocytes.

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**References**


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