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Epithelial Regulation of Eicosanoid Production in Asthma

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Abstract

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 C_4, D_4, and E_4) 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 A_2 ($sPLA_2$) with increased levels in the airways of patients with EIB called $sPLA_2$ group X ($sPLA_2$-X). We have found that $sPLA_2$-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 $sPLA_2$-X. Finally, we demonstrated that $sPLA_2$-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.

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 to 90 minutes 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

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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 $sPLA_2$-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 secreted phospholipase A_2 group X ($sPLA_2$-X) (7), and that $sPLA_2$-X acts on target cells such as eosinophils to initiate cellular eicosanoid synthesis (8). Collectively, these studies identify a novel mechanism linking the airway epithelium to the production of inflammatory eicosanoids by leukocytes in the airways.

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 (10). Hyperpolarized helium images of regional ventilation following exercise challenge demonstrate closure or near closure of segmental airways of the lungs during EIB (11). Bronchoconstriction induced by exercise can be life-threatening as demonstrated by a population-based study that found 61 of 263 sports-related fatalities in young adults were caused by asthma exacerbation during exercise (12). Two recent large cohort studies with long-term longitudinal follow-up demonstrated that exercise-induced wheeze prior to the age of 5 (13) and airway hyperresponsiveness (AHR) to cold dry air hyperpnea in childhood (14) are among the strongest predictors of persistent asthma in adulthood.

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 one second (FEV_1) (2, 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 (F_{ENO}) 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 cysteinyl leukotrienes (CysLTs, LTs C_4, D_4 and E_4) 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 prostaglandins (PG)s 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 A_2 (PLA_2) 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 PGD_2 is largely restricted to myeloid cells, especially mast cells and eosinophils that contain LTC_4 synthase and mast cells that contain PGD_2 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 PGE_2, an eicosanoid produced in large quantities by 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.

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 PGD_2 in the airways following exercise challenge to induce EIB (28, 29). The levels of CysLTs are elevated at 30 min, 1 hour, and 6 hours 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 LTD_4 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 PGE_2 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 PGD_2 in the airways (34), and the mast cell product 9α, 11β-PGF_2 in the urine following exercise challenge (35). The epithelium itself may play a key role in the regulation and production of inflammatory mediators. Following exercise challenge the level of PGE_2 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 triggers 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 towards 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 PGE_2 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 15*S*-Hydroxyeicosatetranoic Acid (15*S* 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 15*S* HETE synthetic pathway, 15-Lipoxigenase-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 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 (29). Following exercise challenge, histamine and the mast cell protease tryptase are released into the airways, and inhibition of EIB with a $CysLT_1$ receptor inhibitor along with an antihistamine reduced the amount of histamine released after exercise challenge (28). In an analogous situation using manitol challenge, pharmacological inhibitors indicate that histamine is responsible for bronchoconstriction early after challenge, while the release of CysLTs is responsible for sustained bronchoconstriction (40).

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 call 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 $CysLT_1$ 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).

Identification of secreted PLA_2 group X ($sPLA_2$-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 PLA_2 enzymes. It is clear from many studies that cytosolic PLA_2 ($cPLA_2\alpha$) 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 $cPLA_2\alpha$, several secreted PLA_2s ($sPLA_2$)s have been shown to significantly increase AA release over $cPLA_2\alpha$ alone, and may preferentially direct eicosanoid

production toward LT synthesis (45). Although the identities of specific $sPLA_2$s were not characterized, increased $sPLA_2$ activity was identified in nasal lavage fluid and in bronchoalveolar lavage (BAL) fluid following allergen challenge (46–48). To determine the identities of the $sPLA_2$s in human airways, we examined induced sputum samples from asthmatics with EIB as well as a non-asthmatic control group and found that $sPLA_2$ groups X and XIIA predominate 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 $sPLA_2$s, groups V and X have generated the most interest because of their capacity to initiate cellular eicosanoid synthesis (49), particularly $sPLA_2$ group X ($sPLA_2$-X) since it is the most potent of the $sPLA_2$s at releasing AA from membrane phospholipids. Because $sPLA_2$-X is able to hydrolyze phosphatidylcholine-rich vesicles at a rate comparable with its action on anionic phospholipids, $sPLA_2$-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 $sPLA_2$-V or $sPLA_2$-X attenuates the development of allergen-induced inflammation, mucus release, and AHR (6, 50), as does inhibition of human $sPLA_2$-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 $sPLA_2$-X protein in induced sputum supernatant and the percentage of epithelial cells immunostaining for $sPLA_2$-X, suggesting that activation or release of $sPLA_2$-X may be involved in the generation of eicosanoids following exercise challenge (3). In subsequent work to better understand the identities of the $sPLA_2$s in human airways, we have found that $sPLA_2$-X and $sPLA_2$-IIA are the predominant $sPLA_2$s in human BAL fluid both in subjects with and without asthma (4). In the airway epithelium, the expression of $sPLA_2$-X predominated (Figure 1), while both $sPLA_2$-X and $sPLA_2$-IIA were expressed in BAL cells (4). The levels of $sPLA_2$-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 $sPLA_2$-X2-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 $sPLA_2$-X in asthma as a regulator of cellular inflammation and eicosanoid formation. Studies are currently underway to better understand the distribution of $sPLA_2$-X expression within the epithelium of patients with asthma and non-asthmatic subjects. It is notable that in the murine model, the expression of $sPLA_2$-X co-localizes to cells expressing MUC5AC suggesting prominent expression in secretory cells such as goblet cells (6).

Transglutaminase 2 (TGM2) is increased in the airways of patients with asthma and regulates the activity of $sPLA_2$-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 NFκB , which induces expression of pro-inflammatory cytokines (54). Using an *in vitro* assay of PLA_2 activity, we found that recombinant human TGM2 enzymatically modifies $sPLA_2$-X leading to a substantial increase in the PLA_2 activity of the enzyme, suggesting that one of the mechanisms of TGM2 action in asthma is regulation of eicosanoid and lysophospholipid synthesis (Figure 2). In a prior investigation, dual inhibitors of TGM2 and $sPLA_2$ 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 PLA_2 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).

Secreted PLA_2 group X ($sPLA_2$-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, LTC_4 synthase, is predominantly present in mast cells and eosinophils in the airways in subjects with asthma (22), and eosinophils are the predominant source of LTC_4 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 $sPLA_2$-X in the airways of patients with EIB and the evidence that eosinophils are a major source of CysLTs, we examined the ability of $sPLA_2$-X to efficiently activate CysLT formation by human eosinophils (8). It is well known that group IVA cytosolic PLA_2 (i.e. $cPLA_2\alpha$) plays a major role in endogenous CysLT synthesis in myeloid cells such as eosinophils (59, 60); however, it is reported that $sPLA_2$ group V ($sPLA_2$-V) initiates CysLT synthesis by human eosinophils in the absence of $cPLA_2\alpha$ activation (61, 62). Although $sPLA_2$-V and $sPLA_2$-X both have high capacity to initiate cellular eicosanoid synthesis (49), $sPLA_2$-V has been difficult to identify in the airways of patients with asthma (4) or EIB (3). To examine the role of $sPLA_2$-X in eosinophil CysLT synthesis, we used recombinant human $sPLA_2$-X to activate human eosinophils isolated from donors with a physician diagnosis of asthma and/or allergy. Exogenous $sPLA_2$-X rapidly caused release of a large portion of labeled AA and CysLT synthesis that was related to the amount of $sPLA_2$-X added exogenously to eosinophils. A specific, active site-directed inhibitor of $sPLA_2$-X inhibited both CysLT synthesis and AA release indicating that $sPLA_2$-X was responsible for the AA release and CysLT synthesis. In addition to AA release, $sPLA_2$-X caused marked lysophospholipid release from eosinophils including LysoPC species known to induce a Ca^{2+} flux in eosinophils. Other lysophospholipid species enriched in AA in human eosinophils, including phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) (63), and plasmenyl PC and PE species (64), were released by the addition of $sPLA_2$-X. Although it is clear that $sPLA_2$-X serves as a major source of AA and lysophospholipids, the mechanism of CysLT formation is more complex. We found that selective inhibitors of $cPLA_2\alpha$ suppressed CysLT formation mediated by $sPLA_2$-X suggesting that $sPLA_2$-X or a product of $sPLA_2$-X activates $cPLA_2\alpha$. Activation of $cPLA_2\alpha$

involves an intracellular calcium flux and phosphorylation of a serine residue by MAP kinases. Treatment with $sPLA_2$-X initiated Ser^{505} -phosphorylation of $cPLA_2\alpha$ and an intracellular Ca^{2+} flux in eosinophils, as well as translocation of $cPLA_2$ and 5-LO to focal locations in the cytoplasm and in the perinuclear space. CysLT formation in response to $sPLA_2$-X was attenuated by pharmacological inhibition of p38 and JNK MAP kinases; further LysoPC initiated CysLT formation that was similarly dependent upon p38 and JNK MAP kinases. Despite the finding of $cPLA_2\alpha$ involvement, it was also apparent that AA release by $sPLA_2$-X may still contribute to additional CysLT synthesis since the addition of $sPLA_2$-X to eosinophils during fMLP-mediated CysLT synthesis further increased CysLT synthesis during these conditions of strong $cPLA_2\alpha$ activation. Thus, we have demonstrated that $sPLA_2$ CysLT synthesis in eosinophils through AA and lysophospholipid release through a mechanism involving $cPLA_2\alpha$ and resulting in the amplification of CysLT synthesis in cells that are actively synthesizing CysLTs induced by another stimulus (Figure 3). These result imply that $sPLA_2$-X mediated activation of eosinophils in the airways may be an important source of CysLTs in asthma.

Conclusions

We have found that EIB is a distinct syndrome in asthma that is related to indirect AHR, and is notable for increased production of CysLTs and shedding of epithelial cells into the airway lumen. Exercise challenge serves as a stimulus to the airway epithelium and adjacent leukocytes resulting in sustained CysLT and PGD_2 release in association with smooth muscle contraction and the release of MUC5AC that may be the consequence of sensory nerve activation. Based on several lines of evidence, mast cells and eosinophils serve as the principal sources of inflammatory eicosanoids in this disorder. Our work in this area led to the identification of $sPLA_2$-X that is strongly expressed in the airway epithelium and is released into the airway lumen in asthma. In a genome-wide expression study, we identified increased TGM2 and demonstrated that TGM2 serves as a regulator of $sPLA_2$-X. Finally, we demonstrated that $sPLA_2$-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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Abbreviations

5-LO	5-Lipoxygenase
15*S*-HETE	15*S*-Hydroxyeicosatetranoic Acid
AA	Arachidonic acid
AERD	Aspirin exacerbated respiratory disease
AHR	Airway Hyperresponsiveness
BAL	Bronchoalveolar Lavage
$cPLA_2\alpha$	Cytosolic Phospholipase $A_2\alpha$
CysLT	Cysteinyl Leukotrienes
CST1	Cystatin 1
ECP	Eosinophilic Cationic Protein

EIB	Exercise-induced Bronchoconstriction
F_{ENO}	Fraction of exhaled nitric oxide
FEV_1	Forced Expiratory Volume in One Second
HIB	Hyperpnea-induced Bronchoconstriction
ICS	Inhaled Corticosteroid
IL-13	Interleukin-13
LT	Leukotriene
MUC5AC	Mucin 5AC
PG	Prostaglandin
PUFA	Polyunsaturated Fatty Acid
PLA_2	Phospholipase A_2
SLPI	Secretory leukocyte peptidase inhibitor
$sPLA_2$	Secreted Phospholipase A_2
TGM2	Transglutaminase 2

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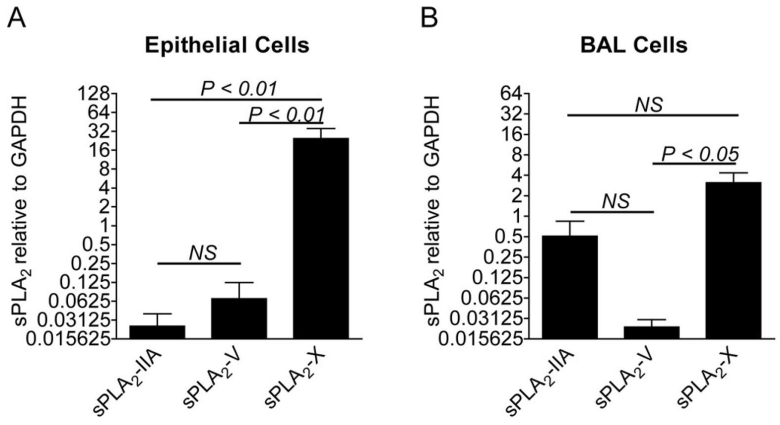

Figure 1. Expression of the $sPLA_2$ enzymes in the airway epithelium and BAL cells

The gene expression of $sPLA_2$-IIA, $sPLA_2$-V, and $sPLA_2$-X was assessed in epithelial brushings and BAL fluid from asthmatic and non-asthmatic subjects. The expression of $sPLA_2$-X was significantly higher than the expression of either $sPLA_2$-V or $sPLA_2$-IIA in the epithelium (**A**). There was no difference in the expression of $sPLA_2$-IIA and $sPLA_2$-V in the epithelium. The expression of $sPLA_2$-X in BAL cells was significantly higher than $sPLA_2$-V, but no different than $sPLA_2$-IIA (**B**). There was no statistically significant difference between the expression of $sPLA_2$ groups IIA and V in BAL cells (Adapted from reference 4).

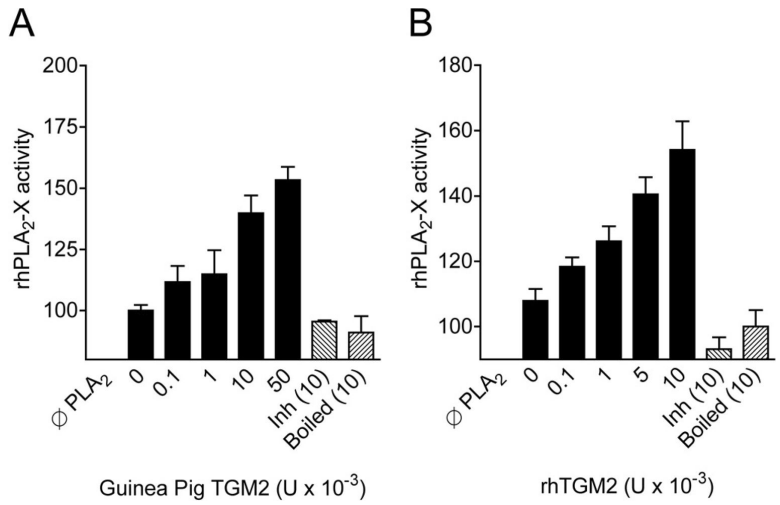

Figure 2. *In vitro* increase in secreted PLA_2 group X activity by TGM2

Pre-incubation of recombinant human $sPLA_2$-X with purified TGM2 from guinea pig liver (**A**) or with recombinant human TGM2 (**B**) causes an increase in the PLA_2 activity of the $sPLA_2$-X enzyme. Denaturing the TGM2 with heat (boiled) or inhibiting the activity of the enzyme by saturating the enzyme with N-carbobenzoxy-Gln-Gly (Inh) demonstrate that the *in vitro* findings are due to the enzymatic activity of TGM2 (Adapted from reference 7).

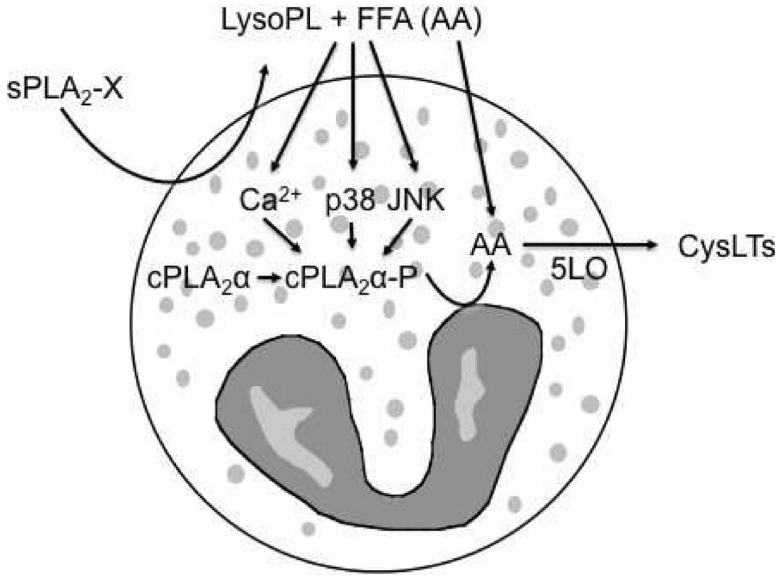

Figure 3. Schematic representation of sPLA$_2$-X-mediated CysLT synthesis by eosinophils

sPLA$_2$-X causes the release of lysophospholipids (*LysoPL*) and free fatty acids (*FFA*), including arachidonic acid (AA) from membrane phospholipids species. sPLA$_2$-X causes CysLT synthesis that is dependent upon cPLA$_2$α a and initiates a Ca^{2+} flux and cPLA$_2$α phosphorylation in eosinophils. We found that the sPLA$_2$-X causes a Ca^{2+} flux and that sPLA$_2$-X- and LysoPC-induced CysLT synthesis could be inhibited by p38 and JNK inhibitors but not by a MEK 1/2 inhibitor. Free AA released by sPLA2-X may contribute to additional CysLT synthesis based on the observation that the addition of sPLA$_2$-X to eosinophils treated with fMLP leads to additional CysLT synthesis (Adapted from reference 8).