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The Intricate Web of Phospholipase A₂s and Specific Features of Airway Hyperresponsiveness in Asthma

To the Editor:

In a recent report, Ueno and colleagues examine the mechanism of exercise-induced bronchoconstriction (EIB) and airway remodeling in mice, strongly implicating leukotriene (LT) biosynthesis (1). The regulation of LT synthesis by PLA₂s (phospholipase A₂s) and the mechanism of EIB are areas that are incompletely understood. The development of features of airway hyperresponsiveness (AHR) and remodeling have been described in high-level/elite athletes, and the model by Ueno and colleagues of repeated high-level exercise in mice nicely models this process and leads to a better understanding of the etiology of AHR in this context. We would like to expound on the forms of AHR, the specific PLA₂s that have been implicated in LT biosynthesis, and the balance of protective versus detrimental effects of exercise in asthma.

There are two general forms of AHR that are pertinent to asthma and serve as the central feature of the disease in most individuals with asthma. Direct or “exogenous” AHR reflects the propensity of the airways to constrict in response to an agent such as methacholine that directly causes bronchoconstriction as was conducted by Ueno and colleagues and is commonly used both as a diagnostic test for asthma in humans (2) and to examine alterations in airway physiology in mouse models of asthma (3). Direct AHR is related to the density of airway smooth muscle in humans (4) and to features of airway geometry, such that individuals with other disorders can have features of direct AHR. In contrast, indirect or “endogenous” AHR reflects the generation of mediators that cause bronchoconstriction in response to common asthma triggers, including dry air challenge either through exercise (i.e., EIB) or eucapnic voluntary hyperpnea, or with an osmotic stimulus such as mannitol or hypertonic saline (5). Allergen-induced bronchoconstriction represents a special form of indirect AHR that requires allergic sensitization (5). Indirect AHR is more specific for asthma and has been related to specific aspects of asthma biology, such as the increased production of cysteinyl LTs (CysLTs; LTs C₄, D₄, and E₄) and mast cell infiltration of the airway epithelium (6, 7). To our knowledge, there is no well-validated mouse model of EIB, but other species such as guinea pigs will develop bronchoconstriction in response to hyperpnea with dry air. The pattern of airway remodeling and increased direct AHR is a pattern

that has been well described and is generally referred to as the injury syndrome (8–11); however, these findings are not synonymous with EIB, and the authors do not examine the mechanism of EIB in the paper.

The authors nicely demonstrate an increase in the levels of CysLTs with regular high-level exercise and a specific role for cytosolic PLA₂ group IVA (cPLA₂-IVα, *Pla2g4*), using an inhibitor that largely targets this enzyme. They also examine the role of one of the secreted PLA₂s (sPLA₂), sPLA₂ group V (sPLA₂-V, *Pla2g5*), that has been well described in mouse models of asthma (12–14). The identification of key sPLA₂s may be critical because it is well known that sPLA₂s can act in conjunction with cPLA₂-IVα to regulate eicosanoid synthesis (15–17). In the study by Ueno and colleagues, the expression and protein levels of sPLA₂-V in the lungs were not increased with regular high-level exercise. After our lab identified elevated levels of CysLTs present in subjects with EIB, we examined a full set of sPLA₂ enzymes initially by semiquantitative methods (18) and then by highly specific methods to resolve the levels of these proteins (19, 20). This work has revealed that, in human airways, the majority of the sPLA₂ activity comes from groups 2A (sPLA₂-IIA, *PLA2G2A*) and 10 (sPLA₂-X, *PLA2G10*) and that the sPLA₂-X protein is increased in the airways of patients with asthma. Although the *PLA2G10* gene is expressed at high levels in the epithelium, it is not differentially expressed in asthma. To provide additional data on the relationship between the levels of sPLA₂-X and different forms of AHR, we used data from a cohort of subjects to quantitatively relate the level of this enzyme in the airways relative to the severity of both direct and indirect AHR (Figure 1).

Another notable finding in the study by Ueno and colleagues is the increase in both cPLA₂-IVα and LTC₄ synthase within the epithelium. Because LTC₄ synthase, which serves to direct eicosanoid synthesis toward CysLTs, is predominantly expressed in leukocytes such as mast cells and eosinophils, and more recently noted in platelets (21) and brush cells (22), it would be helpful to know whether the increase in this enzyme is due to an influx of specific cells into the epithelial layer or if the increase in staining represents epithelial cell immunostaining. This would be of great interest because intraepithelial mast cells are strongly associated with the propensity to develop EIB in response to hyperpnea with dry air, are associated with type 2 inflammation, and can also regulate levels of the *IL33* gene in the epithelium serving as a mechanism to regulate type 2 inflammation (7).

Clearly, more work is needed on the precise cascade of events that are triggered during acute and chronic challenge to the airways by breathing large volumes of dry air. There also needs to be a better understanding of the detrimental and protective effects of exercise in asthma. Ueno and colleagues acknowledge the discordance between their findings and the results from prior studies demonstrating that regular moderate exercise in mice results in markedly attenuated allergen-induced features of airways disease and AHR, possibly through specific regulatory T-cell populations. The editorial accompanying the study by Ueno and colleagues also nicely reviews the correlation between prior murine models and the results from human studies of patients with asthma that demonstrate the benefits of exercise on asthma symptoms, lung function, and airway inflammation (23). It should also be noted that lipid mediators can have both protective as well as detrimental effects (24) in the

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Figure 1. Induced sputum samples were collected at the University of Washington from a study population of nonasthmatic controls and subjects with mild to moderate asthma with and without exercise-induced bronchoconstriction (EIB). The characteristics of this study population and the method of induced sputum sample collection have been previously described (6, 7). Levels of sPLA₂-X (secreted phospholipase A₂ group X) protein in induced sputum were determined by time-resolved fluorescence immunoassay (19), and recombinant human sPLA₂-X was used to generate a linear standard curve (25). (A and B) Baseline induced sputum levels of sPLA₂-X were correlated with direct airway hyperresponsiveness as determined by methacholine challenge (A) and indirect airway hyperresponsiveness as determined by exercise challenge (B). A linear regression line is shown with the 95% confidence interval as dashed lines. AUC30 = the area under the FEV₁ versus postexercise time curve, with greater values indicating more severe EIB; FEV₁ = forced expiratory volume in 1 second; PC₂₀ = the provocative concentration of methacholine that results in a 20% decline in FEV₁ from its baseline value.

airways, suggesting that there may be an ideal amount of exercise to obtain these beneficial effects without the generation of features of airway remodeling. ■

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Reply to: The Intricate Web of Phospholipase A₂s and Specific Features of Airway Hyperresponsiveness in Asthma

From the Authors:

We thank Dr. Murphy and colleagues for taking an interest in our article (1) and their thoughtful comments.

First, we agree with their suggestions regarding airway hyperresponsiveness. In the general murine asthma model, airway resistance (lung resistance) to inhaled methacholine and histamine has been measured, and spontaneous airway hyperresponsiveness cannot be observed (2, 3). Therefore, it

is different from the clinical implications of airway hyperreactivity in asthma. However, we believe our model is a reasonable murine model of exercise-induced bronchoconstriction because it can only be created by long-term strenuous exercise without allergens or airway stimulants, such as tobacco smoke, diesel exhaust particles, and various pathogens.

Regarding PLA₂ (phospholipase A₂), it was not possible to secure a sufficient amount of sample to analyze all members of PLA₂ because of the experimental methods. Based on previous reports (4–6), cPLA₂ (cytosolic PLA₂) group IV and sPLA₂ (secreted PLA₂) group V and group X were analyzed at the protein level. As a result, only cPLA₂ group IV showed a significant change. Regarding the PLA₂ group X, pointed out by Dr. Murphy, the expression levels were too low to present the data in a figure. It is necessary to analyze whether the discrepancy in the data is due to the samples being obtained from mice or the influence of the collected cells, which are tracheal epithelial cells. We also believe this is an important suggestion.

Regarding the expression of LTC₄S (leukotriene C₄ synthase), there was minimal influx of inflammatory cells on immunohistostaining, and there was no clear difference in the LTC₄S-stained portions on inflammatory cells, whereas the changes in bronchial epithelial expression were observed.

Finally, the unique feature of this model is that it is possible to investigate the influences conferred purely by exercises. In clinical cases, there are many biases, such as the complication of conventional asthma, smoking status, and living environment. Therefore, it is difficult to strictly observe changes associated with exercises in human subjects. As Dr. Murphy pointed out, we completely agree that further analysis is needed to elucidate the true effects of exercise on the airway. ■

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