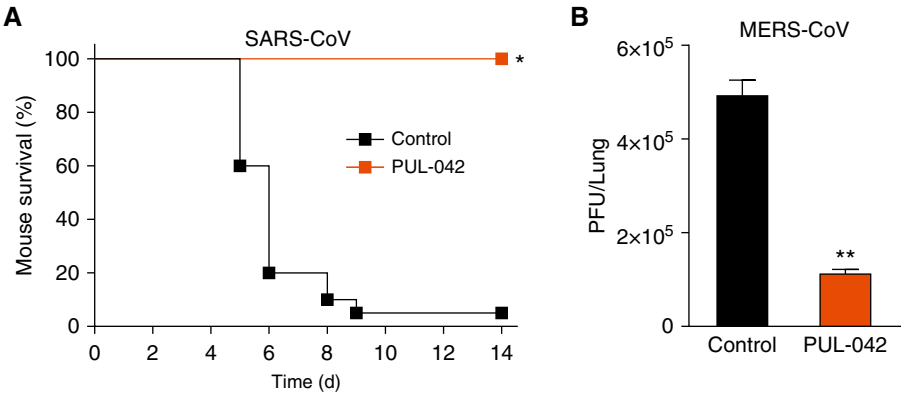

Figure 1. PUL-042 treatment induces resistance against CoV pneumonia. (*A*) Survival of mice challenged with SARS-CoV. $N = 20$ mice/group. (*B*) Lung viral burden of mice 3 days after challenge with MERS-CoV. $N = 5$ mice/group. $^{*}P < 0.00001$ versus sham treated. $^{**}P < 0.0001$ versus sham treated. CoV = coronavirus; MERS-CoV = Middle East respiratory syndrome CoV; PFU = plaque-forming unit; SARS-CoV = severe acute respiratory syndrome CoV.

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COVID-19: Clean up on IL-6

To the Editor:

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents the latest threat to global health security, and the pressure to identify effective therapeutics during this pandemic is immense. This stress has led to the use of unproven therapies with greater than minimal risk. One example is the use of IL-6 receptor antagonists. After an early report of a "cytokine storm" in patients with coronavirus disease (COVID-19), there is increased interest in anti–IL-6 therapy as a treatment option, with ill-defined criteria for use (1).

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The prevailing theory is that SARS-CoV-2 induces the production of cytokines, in particular IL-6, and that these cytokines are a key driver of both lung damage and mortality. However, it is crucial to recognize the uncertainty surrounding the role of IL-6 in viral infections. In experimental viral infection models, IL-6 is a pleiotropic cytokine with complex interactions with multiple signaling cascades, at times producing either proinflammatory or antiinflammatory effects (2). As such, it remains unknown whether elevated IL-6 in viral infections represents a therapeutic target or part of a functioning adaptive immune response.

There has been a long-standing interest in cytokine levels in diverse diseases, including acute respiratory disease syndrome (ARDS) (3). Comparing COVID-19 with our historical knowledge of IL-6 concentrations in other disease states and healthy individuals may be useful. From a study of healthy adult volunteers, IL-6 levels ranged from 1 to 79 pg/ml, with 95% of patients having an IL-6 concentration of less than 42 pg/ml (4). An analysis of patients with ARDS from the SAILS (Statins for Acutely Injured Lungs from Sepsis) study parsed patients into the following two subphenotypes: hyperinflammatory and hypoinflammatory. The hyperinflammatory subphenotype had a median IL-6 concentration of 1,618 pg/ml, whereas the hypoinflammatory group had a median IL-6 concentration of 282 pg/ml (3). Another analysis of serum IL-6 levels reported mean values of 712 pg/ml in patients with ARDS and of 834 pg/ml in patients with severe bacterial pneumonia (5). A COVID-19 cohort in Shanghai had peak elevated IL-6 concentrations ranging from 100 to 200 pg/ml at 20–30 days after symptom onset (6). For comparison, patients who have received chimeric antigen receptor modified T-cell therapy (CAR-T) and developed cytokine release syndrome (CRS) had median IL-6 levels of close to 1,000 pg/ml for grade 4 CRS and above 100 pg/ml for milder grades (7). In light of these data, it appears that patients with SARS-CoV-2 infection have elevated IL-6 levels; however, these are markedly lower than those seen in other severe respiratory diseases or those seen in CAR-T patients with CRS. Although use of blood biomarker levels to direct therapeutic choice seems tempting, the different biology of CAR-T CRS, influenza, and SARS-CoV-2 suggests that a "one cutoff value fits all" approach is inappropriate. In addition, we speculate that the optimal timing of blockade and prolonged IL-6 inhibition is likely disease and IL-6 concentration specific and should be carefully considered for a cytokine with proinflammatory and antiinflammatory effects.

The uncertainties about the use of anti–IL-6 therapy in COVID-19 do not end there. Patients with severe COVID-19 who were administered anti–IL-6 treatments have been reported to have increased secondary infections (8, 9). The side effects of immunomodulatory agents should give pause to off-label use, and they should ideally be administered only as part of a clinical trial. Further data are necessary to determine whether IL-6 is a maladaptive or an adaptive inflammatory factor in COVID-19 pathogenesis. After our initial submission of this letter, Sinha and colleagues published an editorial that the evidence for cytokine storm in COVID-19 pathogenesis is insufficient (3)—we agree and also conclude that the early focus on IL-6 may have cluttered the aisle in our understanding of COVID-19 ARDS pathogenesis. Although a subgroup of patients may benefit from IL-6 blockade, the current data argue against its routine use until more is known. Fortunately, several randomized control trials are underway to determine the efficacy of anti–IL-6 therapy (10).

This pandemic has challenged many of the tenets of good medical practice and intensive care—to first do no harm, to provide safe and evidence-based therapies, and in the absence of other treatments, to provide the best supportive care possible. The threshold for treatment with an unproven therapy should not change based on the novelty of disease and any potential therapies rigorously studied. Otherwise, we will lose the opportunity to learn the most about the disease we are trying so desperately to defeat. ■

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The Intricate Web of Phospholipase A_2s 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 PLA_2s (phospholipase A_2s) 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 PLA_2s 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 C_4, D_4, and E_4) 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 PLA_2 group IVA (cPLA$_2$-IVα, *Pla2g4*), using an inhibitor that largely targets this enzyme. They also examine the role of one of the secreted PLA_2s (sPLA$_2$), sPLA$_2$ group V (sPLA$_2$-V, *Pla2g5*), that has been well described in mouse models of asthma (12–14). The identification of key sPLA$_2$s may be critical because it is well known that sPLA$_2$s can act in conjunction with cPLA$_2$-IVα to regulate eicosanoid synthesis (15–17). In the study by Ueno and colleagues, the expression and protein levels of sPLA$_2$-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 sPLA$_2$ 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 sPLA$_2$ activity comes from groups 2A (sPLA$_2$-IIA, *PLA2G2A*) and 10 (sPLA$_2$-X, *PLA2G10*) and that the sPLA$_2$-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 sPLA$_2$-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 cPLA$_2$-IVα and LTC_4 synthase within the epithelium. Because LTC_4 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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