Response of Lung Microbiota to Changes of Pulmonary Innate Immunity under Healthy Conditions

To the Editor:

We read with much interest the research letter by Pantaleón García and colleagues regarding the response of lung microbiota to changes in pulmonary innate immunity (1). An important question that needs to be addressed is why the lungs of mice were harvested 6 days after exposure to Pam2-ODN, a Toll-like receptor agonist. Inhalation of Pam2-ODN has been shown to protect mice infected with a virus or bacteria a few hours after exposure to the Toll-like receptor agonist (2–4). Therefore, it is conceivable that earlier evaluation of lung microbiota would have provided different results. On the other hand, previous studies have shown that multisource reactive oxygen species generation is required to protect mice against viral or bacterial infection after exposure to Pam2-ODN (5). In addition, another study has shown that exposure to Pam2-ODN is associated with a dramatic increase in the expression of inflammatory cytokines (e.g., tumor necrosis factor-α) and chemokines (e.g., Cxcl1, Cxcl2, Cxcl13) in the lungs (6). These observations suggest that measuring reactive oxygen species, inflammatory cytokines, or chemokines in blood or lung homogenates may provide key information to determine the optimal time to assess changes in lung microbiota in response to an enhanced pulmonary innate immunity. We believe that addressing the above questions may further clarify whether changes in pulmonary innate immunity affect lung microbial communities. ■

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Reply to Yasuma *et al.*

From the Authors:

We appreciate the thoughtful letter from Yasuma and colleagues regarding our recently published research letter (1).

The authors inquire about our selection of time points and speculate that had we compared lung microbiota at an earlier time point after exposure, we might have observed an effect of innate immune modulation on lung communities. We chose our time point (harvest 6 d after exposure) based on prior work characterizing the sustained effects of a single Pam2-ODN exposure on lung immunity, reflected in persistent protection from bacterial, fungal, and viral infection (2). We agree with the authors that our findings do not exclude the possibility of a transient effect and stated as much in our original manuscript: "Although we detected no appreciable effect of Pam2-ODN on lung bacterial communities 6 days after exposure, it is entirely possible that TLR agonism has a short-lived effect on lung microbiota that was resolved by the time of harvest due to the continuous exposure of the lungs to environmental microbiota" (3).

To directly address this possibility, we performed a separate experiment to determine the effect of lung innate immune modulation (via single Pam2-ODN exposure) on lung bacterial communities at 48 hours after exposure. Experimental conditions, interventions, and measurements were otherwise identical to those

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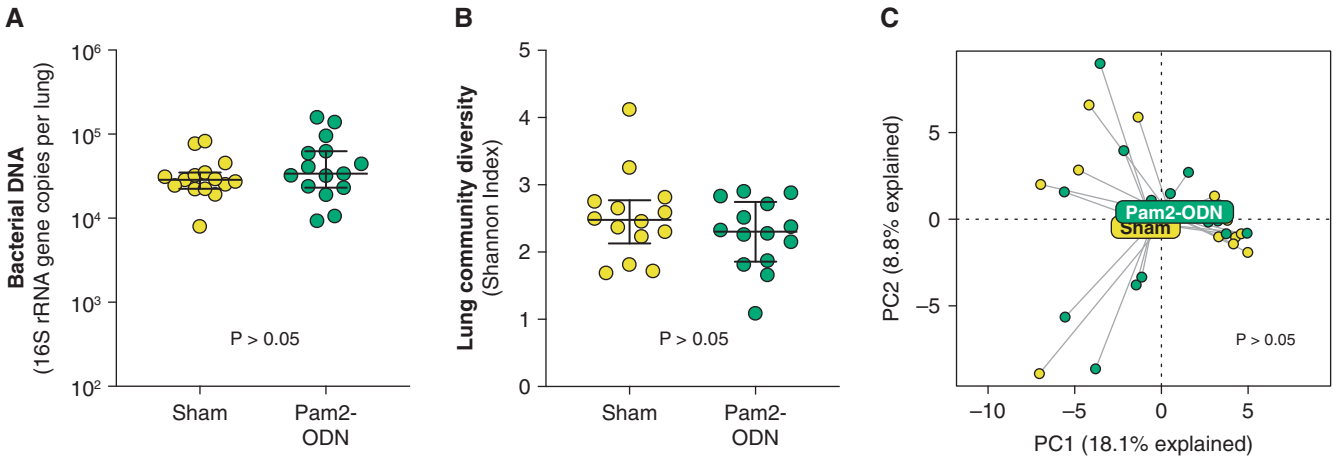

Figure 1. Experimental modulation of lung innate immune tone does not influence lung microbial communities at 48 hours. Healthy, adult mice received either phosphate-buffered saline inhalation ("sham") or synergistic TLR2/6 and TLR9 stimulation via inhaled Pam2-ODN. Lungs were harvested 48 hours after exposure, and lung microbiota were characterized via (*A*) droplet digital PCR or (*B* and *C*) 16S rRNA gene amplicon sequencing. TLR agonism did not influence (*A*) the total bacterial burden in murine lungs, (*B*) lung bacterial diversity, or (*C*) lung community composition. Horizontal lines and error bars represent median and interquartile range, respectively. Significance was determined using (*A*) Mann-Whitney test, (*B*) Student's *t* test, and (*C*) permutational multivariate ANOVA. $n = 15$ mice per experimental group; one specimen per group was excluded from diversity and community composition analysis because of inadequate sequencing depth. PC = principal component; rRNA = ribosomal RNA.

described in our research letter (1). As demonstrated in Figure 1, we observed no effect of Pam2-ODN exposure on lung bacterial density, diversity, or community composition at 48 hours. We believe these findings do not support the authors' hypothesis and further confirm our interpretation that innate immune modulation (via TLR2/6 and TLR9 agonism) has no appreciable effect on the lung microbiota of healthy mice.

We note that in the relevant study by Wu and colleagues (4) (to which our research letter was a response), a single experimental modulation of lung microbiota resulted in persistent immune effects detectable at 14 days. Thus, even if selective innate immune modulation *does* transiently influence lung microbiota (i.e., for hours rather than days), its effect is more self-limited than the converse interaction. As stated in our research letter, we believe important unanswered questions remain regarding *1*) timing and duration of lung innate immune modulation and its potential effect on lung microbiota, *2*) the effect of other specific immune-modulating exposures (e.g., TLR inhibition, agonism of other TLRs) on lung microbiota, and *3*) the relative role of immune tone in shaping lung communities in disease states (as opposed to health).

Although we appreciate the authors' summary of several mechanistic effects of synergistic TLR agonism (5, 6), we do not believe these observations alter our interpretation of our microbiome findings. Although indices of lung inflammation have been correlated with lung microbiota in both health and disease (3, 7), the causal direction underlying this correlation has not been fully elucidated. To our knowledge, no study to date has demonstrated that host-derived reactive oxygen species or cytokines exert a causal influence on lung microbial communities (as postulated by the authors). We believe that, taken with the findings of Wu and colleagues (4), our findings suggest that in health, variation in lung immunity reflects variation in lung microbiota, rather than vice versa. Yet we readily concede that considerable additional work will be necessary to definitively resolve this question. ■

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Time to Tailor the One-Size-Fits-All Approach?

To the Editor:

We read with interest the study by Sinha and colleagues in a recent issue of the *Journal* (1) examining the latent classes of coronavirus disease (COVID-19)–associated acute respiratory distress syndrome (ARDS). The authors concluded that COVID-19–associated ARDS can be classified, like other causes of ARDS, into a hypoinflammatory and hyperinflammatory subphenotype. Both subphenotypes appear to have distinct outcomes, with increased mortality in the hyperinflammatory subgroup. In addition to these more generalizable effects in ARDS, this study also reveals more COVID-19–associated ARDS-specific findings. In particular, the viral load at the start of treatment influences the outcome, especially in patients with a hypoinflammatory subphenotype treated with corticosteroids with a high viral load, expressed as a low cycle threshold (CT) value. A delayed viral clearance was suggested as the underlying cause of the negative effects of corticosteroids in this group. Detrimental effects of corticosteroids on viral clearance are well known and have been described in multiple viral infections (i.e., severe acute respiratory syndrome coronavirus 1 and influenza). In addition, there is mounting evidence that secondary infections (e.g., COVID-19–associated pulmonary aspergillosis) are increased since the introduction of routinely starting corticosteroids in the treatment of COVID-19–associated ARDS (2, 3).

Given the many potential drawbacks of corticosteroids in the treatment of COVID-19–associated ARDS, there is an increased demand for personalized care in these patients, because one size may not fit all (4). Personalized or tailored medicine targeting the different ARDS phenotypes was suggested several years ago as an option to improve survival. Further understanding of the heterogeneity in the molecular, mechanical, and inflammatory response underlying the ARDS pathogenesis is an essential step to enable this personalized therapy.

How can we use the study of Sinha and colleagues to further personalize the therapy in daily care? First, we will have to be able to identify the two subphenotypes of ARDS. Ideally, we would apply classes from the latent class analysis to our patients. However, several of the parameters used are only available in a research setting and not readily available in daily practice. A suitable alternative may be to use a clinical classifier model that has also been studied and has a good correlation with the latent class analysis in ARDS of COVID-19 and non–COVID-19 origin (5). The top 10 criteria from this model (bicarbonate, vasopressor use, creatine, bilirubin, and albumin levels, heart rate, $\dot{V}_E$, platelet count, systolic blood pressure, and white blood cell count) are readily available and adequately differentiate between subphenotypes. Once this distinction in subphenotypes is established, a decision may be made regarding the initiation of corticosteroid therapy if the viral load is additionally factored in the decision. Readily available CT values of RT-PCR seem to be a reasonable derivative in this respect, although absolute values cannot be given because the quantified degrees of PCR-CT and viral loads are inconsistently defined across assays (6).

With parameters available almost everywhere, we may distinguish ARDS subphenotypes in COVID-19. Let us look carefully at the individual patient with COVID-19 in the ICU and prepare a tailored therapeutic strategy that may or may not include the use of steroids. ■

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