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.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Taro Yasuma, M.D.
Corina N. D’Alessandro-Gabazza, D.M.D.
Hajime Fujimoto, M.D.
Tetsu Kobayashi, M.D.
Esteban C. Gabazza, M.D., Ph.D.*
Mie University
Tsu, Japan

ORCID ID: 0000-0001-5748-1499 (E.C.G.).

*Corresponding author (e-mail: gabazza@doc.mediec.mie-u.ac.jp).

References

1. Pantaleón García J, Hinkle KJ, Falkowski NR, Evans SE, Dickson RP. Selective modulation of the pulmonary innate immune response does not change lung microbiota in healthy mice. Am J Respir Crit Care Med 2021;204:734–736.

2. Cleaver JO, You D, Michaud DR, Pruneda FA, Juarrez MM, Zhang J, et al. Lung epithelial cells are essential effectors of inducible resistance to pneumonia. Mucosal Immunol 2014;7:78–88.

3. Kirkpatrick CT, Wang Y, Leiva Juarrez MM, Shrivshankar P, Pantaleón García J, Plumer AK, et al. Inducible lung epithelial resistance requires multisource reactive oxygen species generation to protect against viral infections. Mbio 2018;9:e00696-18.

4. Wali S, Flores JR, Jaramillo AM, Goldblatt DL, Pantaleón García J, Tuvi M, et al. Immune modulation to improve survival of viral pneumonia in mice. Am J Respir Cell Mol Biol 2020;63:758–766.

5. Ware HH, Kulkami VV, Wang Y, Pantaleón García J, Leiva Juarrez M, Kirkpatrick CT, et al. Inducible lung epithelial resistance requires multisource reactive oxygen species generation to protect against bacterial infections. PLoS One 2019;14:e0208216.

6. Tuvi M, Gilbert BE, Dickey BF, Evans SE. Synergistic TLR2/6 and TLR9 activation protects mice against lethal influenza pneumonia. PLoS One 2012;7:e30596.
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 recent 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.

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.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Jezreel Pantaleon García, M.D.
University of Texas MD Anderson Cancer Center
Houston, Texas

Kevin J. Hinkle, B.S.
Nicole R. Falkowski, M.S.
University of Michigan Medical School
Ann Arbor, Michigan

Scott E. Evans, M.D.*
University of Texas MD Anderson Cancer Center
Houston, Texas

Robert P. Dickson, M.D.‡
University of Michigan Medical School
Ann Arbor, Michigan

and

Michigan Center for Integrative Research in Critical Care
Ann Arbor, Michigan

ORCID IDs: 0000-0002-9647-2584 (J.P.G.); 0000-0003-4503-0644 (S.E.E.); 0000-0002-6875-4277 (R.P.D.).

*Co–senior authors.
‡Corresponding author (e-mail: rodickso@med.umich.edu).

References
1. Pantaleon García J, Hinkle KJ, Falkowski NR, Evans SE, Dickson RP. Selective modulation of the pulmonary innate immune response does
not change lung microbiota in healthy mice. Am J Respir Crit Care Med 2021;204:734–736.
2. Evans SE, Tuvim MJ, Fox CJ, Sachdev N, Gibiansky L, Dickey BF. Inhaled innate immune ligands to prevent pneumonia. Br J Pharmacol 2011;163:195–206.
3. Dickson RP, Erb-Downward JR, Falkowski NR, Hunter EM, Ashley SL, Huffnagle GB. The lung microbiota of healthy mice are highly variable, cluster by environment, and reflect variation in baseline lung innate immunity. Am J Respir Crit Care Med 2018;198:497–508.
4. Wu BG, Sulaiman I, Tsay JJ, Perez L, Franca B, Li Y, et al. Episodic aspiration with oral commensals induces a myD88-dependent, pulmonary T-helper cell type 17 response that mitigates susceptibility to Streptococcus pneumoniae. Am J Respir Crit Care Med 2021;203:1099–1111.
5. Ware HH, Kulkarni VV, Wang Y, Pantaleón García J, Leiva Juarez M, Kirkpatrick CT, et al. Inducible lung epithelial resistance requires multisource reactive oxygen species generation to protect against bacterial infections. PLoS One 2019;14:e0208216.
6. Tuvim MJ, Gilbert BE, Dickey BF, Evans SE. Synergistic TLR2/6 and TLR9 activation protects mice against lethal influenza pneumonia. PLoS One 2012;7:e30596.
7. Ashley SL, Sjoding MW, Popova AP, Cui TX, Hoostal MJ, Schmidt TM, et al. Lung and gut microbiota are altered by hyperoxia and contribute to oxygen-induced lung injury in mice. Sci Transl Med 2020;12:eaauf9959.

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, V̇s, 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.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Marnix Kuindersma, M.D.*
Gelre Hospitals
Apeldoorn, the Netherlands

Peter E. Spronk, M.D., Ph.D.
Gelre Hospitals
Apeldoorn, the Netherlands

and

Expertise Center for Intensive Care Rehabilitation Apeldoorn
Apeldoorn, the Netherlands

*Corresponding author (e-mail: m.kuindersma@gelre.nl).

References

1. Sinha P, Furfaro D, Cummings MJ, Abrams D, Delucchi K, Maddali MV, et al. Latent class analysis reveals COVID-19–related acute respiratory distress syndrome subgroups with differential responses to corticosteroids. Am J Respir Crit Care Med 2021;204:1274–1285.