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.

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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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