

Correspondence

Reply

To the Editor:

We thank Ling et al¹ for their interest in our report² of the characterization and interpretation of bacterial microbiota detected in adults with severe asthma. They raise 2 overarching questions.¹ The first is our focus on bacteria represented in protected bronchial brushings and not nasopharyngeal microbiota, which they argue might be a significant determinant of risk for “bacterial invasion” into the lower airways. Our view is that the mucosal-epithelial interface in the bronchial tree is the most relevant site for first examination of microbial-host associations in asthma, a disease of the subglottic airways. Upon identification of such associations in the bronchial airways, we would agree that it is of subsequent interest to investigate whether the nasopharynx could be a relevant source population of microbes. In children, sampling of the lower airways is more challenging and thus study of upper airway microbiota can and has been informative for particular research questions. Recent studies have contributed concerted efforts to understanding the relationship between upper and lower respiratory bacterial microbiota composition.^{3,4} One such study applied the neutral model of community ecology to assess structuring of the lung microbiome in adults.⁴ Although distance from a potential source community is one determinant of bacterial community similarity between biogeographically separated sites, investigators have found that neutral distribution of microbes distinguishes a healthy lung microbiome from that seen in patients with pulmonary disease. That is, differential growth of particular bacteria (deviation from the neutral model) is seen in the lungs of patients with pulmonary disease.

Ling et al’s second question relates to approaches for characterizing bacterial community composition and assessing interindividual microbiota variability, which has been reported in all human niches studied to date, including the respiratory tract.^{3,5,6} We take exception to their inaccurate summary of the approaches we have applied. To deeply characterize bronchial bacterial composition, we used a hybridization-based, high-probe density, bacterial DNA microarray,⁷ a well-described platform that assays in parallel and simultaneously for all communities present in a sample. This technologic approach, notwithstanding updates in reference databases, is not subject to the particular issue that arises and is of concern when relatively shallow depths of sequencing effort are achieved on next-generation sequencing platforms, which could result in insufficient microbial community profiling. We are particularly puzzled by their statement that

“diversity analysis seemed to be ignored” in our study. We indeed examined diversity and related community metrics in our analyses. As stated or illustrated in the article, beta-diversity measures (Bray-Curtis or Unifrac distances) were calculated and used in analyses that identified certain phenotypic features to be differentially associated with patterns of bacterial composition among subjects. We also observed and report correlations between alpha diversity (inverse Simpson and Faith’s phylogenetic diversity) and epithelial expression of *FKBP5* and measures of asthma control. Indeed, these measures of bronchial bacterial composition underlie several of our key findings.

Yvonne J. Huang, MD^a
Joseph R. Arron, MD, PhD^b
Homer Boushey, MD^c

From ^athe Division of Pulmonary/Critical Care Medicine, University of Michigan Health System, Ann Arbor, Mich; ^bGenentech Inc, South San Francisco, Calif; and ^cthe Division of Pulmonary/Critical Care Medicine, University of California San Francisco, Calif. E-mail: yvjhuang@umich.edu.

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