Latin American Thoracic Association. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: treatment of idiopathic pulmonary fibrosis: an update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med* 2015;192:e3–e19.
5. Nghiem AZ, Mahmoud Y, Som R. Evaluating the quality of internet information for breast cancer. *Breast* 2016;25:34–37.
6. Albright K, Walker T, Baird S, Eres L, Farnsworth T, Fier K, Kervitsky D, Korn M, Lederer DJ, McCormick M, *et al*. Seeking and sharing: why the pulmonary fibrosis community engages the web 2.0 environment. *BMC Pulm Med* 2016;16:4.

The Lung Microbiome in HIV
Getting to the HAART of the Host–Microbe Interface

Widespread use of highly active antiretroviral therapy (HAART) radically altered the natural history of HIV infection from that of a rapidly progressing disease to that of a chronic, treatable condition. Yet HIV-infected patients continue to suffer increased mortality from a variety of respiratory pathologies, both infectious and otherwise (1). Initial investigations into this susceptibility focused largely on host immunity and cellular function (2), overlooking the potential contribution of respiratory microbiota. In 2009, prompted by the revolution in culture-independent microbiology, the National Institute of Health launched the LHMP (the Lung HIV Microbiome Project), a research consortium charged with identifying the role of respiratory microbiota in the pathogenesis of HIV-related lung disease.

The LHMP consortium has since shared two key findings on this subject, one surprisingly positive and one surprisingly negative. The surprisingly positive finding, reported in the *Journal* in 2013 (3), was the frequent detection of *Tropheryma whipplei* in the lungs of HIV-infected individuals. This bacterium, the etiologic agent of Whipple's disease, had not previously been reported in the lung. The surprisingly negative finding, recently reported by Beck and colleagues in the *Journal* (4), was that, in a broader comparison of the respiratory microbiota of HIV-positive versus HIV-negative subjects, no significant differences were detected in lung communities.

However, in this issue of the *Journal*, Twigg and colleagues (pp. 226–235) report significant differences in the lung microbiota of 30 subjects with advanced HIV compared with the microbiota of 22 HIV-negative subjects (5). How can we reconcile the apparently discordant results of these two studies? A partial answer surely lies in key clinical differences between the cohorts. The HIV-positive population studied by Beck and colleagues was relatively healthy; even the HAART-naive patients had a median serum CD4 count of 668/μL. In contrast, the HIV-positive population in the current study had markedly fewer CD4 cells and higher serum viral RNA levels. It is certainly clinically and biologically plausible that changes in microbiota are related to the severity of the host's immune dysfunction.

A conspicuous difference in the two studies is the use of whole bronchoalveolar lavage (BAL) fluid (used by Beck and colleagues) and acellular BAL fluid (used in the current study and obtained via a low-speed, short-duration centrifugation step that separates the lavaged fluid from eukaryotic host cells). A head-to-head comparison of bacteria detected in whole and acellular BAL fluid revealed that the cell removal step significantly decreases the yield of bacterial amplification, reduces the community richness of BAL microbiota, and selectively alters taxonomic composition (6). Concordance of bacterial communities in paired specimens was strongly correlated with bacterial burden: the less bacterial DNA present, the greater the effect cell removal had on identified communities. By all available evidence, the cell removal step may render the bacterial biomass in BAL fluid, which was already low, even lower. This is perhaps not surprising, as some undetermined fraction of lung microbiota is certain to be host cell-associated, via biofilms, cell adhesion, or intracellular location.

Does this mean that acellular BAL is of zero value in the study of the lung microbiome? Of course not. Although bacterial communities detected in whole and acellular BAL fluid were distinct, they were not unrelated (6). The composition of the lung microbiome, as detected in acellular BAL fluid, is significantly correlated with important features of the alveolar inflammatory response (7, 8), and in the current study, it is correlated with disease state (HIV) and treatment (HAART) (5). Indeed, concurrent use and comparison of whole and acellular BAL may prove to be a useful means of localizing the anatomical compartments of detected microbiota (6).

Importantly, the limitations of all BAL specimens, both whole and acellular alike, demand special caution in their use and interpretation. The field has grown increasingly aware that the "sterile" reagents used in specimen collection, DNA isolation, and sequencing contain bacterial DNA capable of contaminating sequencing runs (and confounding investigators) (9). This issue is intrinsic to all low-biomass microbiome studies. As an example, *Flavobacterium* sp., a bacterial group strongly enriched in the acellular BAL specimens of healthy subjects in the current study, has been shown to be present in the reagents found in DNA isolation kits (9). Thus, the source and significance of this bacteria cannot be determined in isolation. Acellular BAL fluid, similar to whole BAL fluid and all low-biomass specimens, should be sequenced and analyzed alongside numerous "negative" control specimens representing plausible sources of environmental or procedural contamination. Contamination of the bronchoscope by pharyngeal microbiota, once a matter of great debate, has not been shown to be a major and frequent event that should preclude use of bronchoscopy to sample the lower airways (10). The recurrent finding of taxa commonly found in the upper airways in multiple studies is not unexpected, considering the anatomical continuity of the upper and lower airways and the frequency of subclinical aspiration in health and disease (11, 12).

A major advancement of the current study is that subjects with HIV underwent prospective lower airway sampling. Importantly, this design allowed the investigators to demonstrate a convincing relationship between HAART-induced immune reconstitution and the

Research support provided by grants K23 AI102970 (L.N.S.), R01 HL125816 (L.N.S.), K23 HL130641 (R.P.D.), and UL1TR000433 (R.P.D.).

composition of the acellular BAL microbiome. The increase in *Prevotella* spp. and *Veillonella* spp. during HAART-induced immune reconstitution is provocative. When previously observed in the lung, these taxa (both common oral commensals) have had consistent associations with lung health (10, 13), and their relative abundance has been positively associated with enhanced alveolar inflammation (7, 8). Although it is tempting to speculate that the enrichment with these microorganisms observed during HAART-induced immune reconstitution may contribute to a pro-inflammatory state involved in chronic lung pathology, we do not yet know whether this change in immune tone is protective, pathogenic, or both.

The next step in our understanding of the lung microbiome will be determining how it intersects with the host immune response and disease pathogenesis. The only way to accomplish this will be by summoning a variety of complementary approaches: metagenomics, transcriptomics, and metabolomics to determine function; animal models to identify mechanism; longitudinal human studies to understand stability and natural history; and clinical trials to identify therapeutic targets. The current study is an important step in this direction. ■

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

Leopoldo N. Segal, M.D., M.S.
Division of Pulmonary and Critical Care Medicine
New York University School of Medicine
New York, New York

Robert P. Dickson, M.D.
Division of Pulmonary and Critical Care Medicine
University of Michigan Medical School
Ann Arbor, Michigan

ORCID IDs: 0000-0003-3559-9431 (L.N.S.); 0000-0002-6875-4277 (R.P.D.).

References

1. Crothers K, Huang L, Goulet JL, Goetz MB, Brown ST, Rodriguez-Barradas MC, Oursler KK, Rimland D, Gibert CL, Butt AA, *et al*. HIV infection and risk for incident pulmonary diseases in the combination antiretroviral therapy era. *Am J Respir Crit Care Med* 2011;183:388–395.
2. Hirschtick RE, Glassroth J, Jordan MC, Wilcosky TC, Wallace JM, Kvale PA, Markowitz N, Rosen MJ, Mangura BT, Hopewell PC; Pulmonary Complications of HIV Infection Study Group. Bacterial pneumonia in persons infected with the human immunodeficiency virus. *N Engl J Med* 1995;333:845–851.
3. Lozupone C, Cota-Gomez A, Palmer BE, Linderman DJ, Charlson ES, Sodergren E, Mitreva M, Abubucker S, Martin J, Yao G, *et al*.; Lung HIV Microbiome Project. Widespread colonization of the lung by *Tropheryma whipplei* in HIV infection. *Am J Respir Crit Care Med* 2013;187:1110–1117.
4. Beck JM, Schloss PD, Venkataraman A, Twigg H III, Jablonski KA, Bushman FD, Campbell TB, Charlson ES, Collman RG, Crothers K, *et al*.; Lung HIV Microbiome Project. Multicenter comparison of lung and oral microbiomes of HIV-infected and HIV-uninfected individuals. *Am J Respir Crit Care Med* 2015;192:1335–1344.
5. Twigg HL III, Knox KS, Zhou J, Crothers KA, Nelson DE, Toh E, Day RB, Lin H, Gao X, Dong Q, *et al*. Effect of advanced HIV infection on the respiratory microbiome. *Am J Respir Crit Care Med* 2016;194: 226–235.
6. Dickson RP, Erb-Downward JR, Prescott HC, Martinez FJ, Curtis JL, Lama VN, Huffnagle GB. Cell-associated bacteria in the human lung microbiome. *Microbiome* 2014;2:28.
7. Segal LN, Alekseyenko AV, Clemente JC, Kulkarni R, Wu B, Gao Z, Chen H, Berger KI, Goldring RM, Rom WN, *et al*. Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation. *Microbiome* 2013;1:19.
8. Segal LN, Clemente JC, Tsay J-CJ, Koralov SB, Keller BC, Wu BG, Li Y, Shen N, Ghedin E, Morris A, *et al*. Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol* 2016;1:16031.
9. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol* 2014;12:87.
10. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. *Annu Rev Physiol* 2016;78:481–504.
11. Gleeson K, Eggli DF, Maxwell SL. Quantitative aspiration during sleep in normal subjects. *Chest* 1997;111:1266–1272.
12. Huxley EJ, Viroslav J, Gray WR, Pierce AK. Pharyngeal aspiration in normal adults and patients with depressed consciousness. *Am J Med* 1978;64:564–568.
13. Molyneaux PL, Mallia P, Cox MJ, Footitt J, Willis-Owen SAG, Homola D, Trujillo-Torralbo M-B, Elkin S, Kon OM, Cookson WOC, *et al*. Outgrowth of the bacterial airway microbiome after rhinovirus exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013;188:1224–1231.

Opening the Debate on the New Sepsis Definition

Precision Medicine: An Opportunity to Improve Outcomes of Patients with Sepsis

Clinical outcomes from sepsis have markedly improved over the last 2 decades, facilitated in part by the development in 1992 (and refinement in 2001) of consensus definitions (1–3). Mortality has decreased, and earlier recognition and treatment of sepsis are now standard of care. Nevertheless, major challenges remain. Sepsis contributes to at least 1 of every 3 deaths in hospitalized patients in the United States (4), in addition to its major global impact on mortality (5), and many survivors are plagued by functional and cognitive limitations with long-lasting repercussions (6). Furthermore, we still lack any specific pharmacotherapies for sepsis, pointing to a major unmet need for new approaches.

The new consensus definitions published February 2016 in *JAMA* will certainly have an important impact on epidemiologic studies of sepsis, as did their predecessors (7). In addition, as the new definitions incorporate a requirement for organ dysfunction, their application in clinical trials may result in prognostic enrichment—that is, enrollment of more severely ill patients who

Supported by National Institutes of Health grant HL131621.