9. Wootton SC, Kim DS, Kondoh Y, Chen E, Lee JS, Song JW, *et al*. Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:1698–1702.
10. Drake TM, Docherty AB, Harrison EM, Quint JK, Adamali H, Agnew S, *et al*.; ISARIC4C Investigators. Outcome of hospitalization for COVID-19 in patients with interstitial lung disease: an international multicenter study. *Am J Respir Crit Care Med* 2020;202:1656–1665.
11. Esposito AJ, Menon AA, Ghosh AJ, Putman RK, Fredenburgh LE, El-Chemaly SY, *et al*. Increased odds of death for patients with interstitial lung disease and COVID-19: a case–control study [letter]. *Am J Respir Crit Care Med* 2020;202:1710–1713.
12. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. *Eur Respir J* 2011;37:356–363.
13. Wong AW, Fidler L, Marcoux V, Johannson KA, Assayag D, Fisher JH, *et al*. Practical considerations for the diagnosis and treatment of fibrotic interstitial lung disease during the coronavirus disease 2019 pandemic. *Chest* 2020;158:1069–1078.
14. Ryerson CJ, Berkeley J, Carrieri-Kohlman VL, Pantilat SZ, Landefeld CS, Collard HR. Depression and functional status are strongly associated with dyspnea in interstitial lung disease. *Chest* 2011;139:609–616.
15. Gries CJ, Bhadriraju S, Edelman JD, Goss CH, Raghu G, Mulligan MS. Obese patients with idiopathic pulmonary fibrosis have a higher 90-day mortality risk with bilateral lung transplantation. *J Heart Lung Transplant* 2015;34:241–246.
16. Moor CC, Mostard RLM, Grutters JC, Bresser P, Aerts JGJV, Chavannes NH, *et al*. Home monitoring in patients with idiopathic pulmonary fibrosis: a randomized controlled trial. *Am J Respir Crit Care Med* 2020;202:393–401.

In Pursuit of Microbiome-based Therapies for Acute Respiratory Failure

A presumably overly robust inflammatory response has been associated with poor clinical outcomes in patients with acute respiratory failure, including patients with acute respiratory distress syndrome (ARDS) and sepsis (1). Likewise, both abnormal gut and respiratory microbiota patterns (termed "dysbiosis") are also predictive of increased mortality among critically ill patients (2). The ambitious aim of the study by Kitsios and colleagues (pp. 1666–1677) in this issue of the *Journal* is to better define the interplay between the host inflammatory response and the lung microbiome and the impact of this relationship on clinical outcomes in a heterogenous population of critically ill patients with acute respiratory failure (3). The results of this investigation represent an important step in the process of developing a microbiome-guided or microbiome-based treatment for critically ill patients with acute respiratory failure.

The cohort characteristics in the study by Kitsios and colleagues were typical of an ICU population of patients with acute respiratory failure requiring mechanical ventilation: extrapulmonary sepsis (18%), ARDS (24%), and pneumonia (40%) were common diagnoses, and 32% of the patients received antibiotics before admission to the ICU. At the time of enrollment (<72 h postintubation), posterior oropharyngeal swab and endotracheal aspirate (ETA) samples were collected and analyzed with 16s ribosomal RNA gene sequencing to characterize the microbiota of these respective environments. Simultaneously, the following plasma inflammation–related biomarkers were measured: receptor of advance glycation end-products, soluble tumor necrosis factor receptor 1, IL-10 fractalkine, and angiopoietin 2. This biomarker data was used in conjunction with clinical variables to dichotomize the patients into either hyper- (23%) or hypoinflammatory (77%) phenotypes. Similar to prior, albeit smaller, microbiome studies of the critically ill, the upper and lower respiratory microbiota demonstrated reduced α and β diversity when compared with samples from healthy control subjects (4). Nonetheless, there was substantial heterogeneity in bacterial composition across samples from study patients, which was addressed by using Dirichlet-multinomial models and Laplace approximation of model fitting to identify distinct microbial clusters among the upper and lower respiratory samples. Common to both the upper and lower respiratory sampling was a particular cluster ("cluster 2") that was notable for having a lower α diversity and a high abundance of respiratory pathogens. In particular, cluster 2 samples found in lower respiratory samples demonstrated a high abundance of *Staphylococcus*, *Stenotrophomonas*, *Enterobacteriaceae*, and *Pseudomonadaceae* and a low abundance of oral-origin organisms associated with a healthy lung microbiome. In addition, cluster 2, especially in ETA samples, was noted to have a number of clinical associations, most of which were unfavorable. Patients with ETA cluster 2 were more likely to have chronic obstructive pulmonary disease at baseline, were more likely to have been treated with antibiotics before being admitted to the ICU, and were more likely to be diagnosed with ARDS and extrapulmonary sepsis. The hyperinflammatory subphenotype was also more prevalent among patients with cluster 2–enriched ETA samples (odds ratio, 1.2 [1.1–1.9]; $P = 0.03$, adjusted for antibiotic exposures). Unsurprisingly, patients afflicted within cluster 2 microbiota suffered comparatively worse outcomes, including a higher 30-day mortality. The authors then used these results to construct a dysbiosis index based on the relative abundance of protective microbiota (≥30%) and α diversity (Shannon index ≥ 1.98) that was predictive of both a hyperinflammatory state and an increased mortality rate.

The limitations of this study are worth noting but do not detract significantly from the overall results. It was a single-center trial (microbiomes are known to vary based on geography), convenience sampling was employed, and enrollment was

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slow occurring over a 45-month time period. The study samples were collected at only one time point and thus do not provide insight into dynamic changes occurring in the microbiome over the course of an ICU admission; also, unfortunately, paired gut microbiome sampling was not performed (the gut microbiome influences the respiratory microbiome) (5). These shortcomings aside, the results of this study provide guidance as to how the field should proceed in pursuit of better therapies for acute respiratory failure. First, these results confirm the findings of other, smaller studies: a lung ecosystem that lacks diversity and is dominated by pathogens and relatively devoid of protective organisms is associated with a poor clinical outcome in patients with acute respiratory failure. Second, the authors build on their prior work defining a hyperinflammatory state that is associated with worse clinical outcomes in ARDS and show that this phenotype is also associated with dysbiosis and higher mortality in a heterogeneous critically ill population with acute respiratory failure (6).

Despite decades of research and clinical trials, setting aside low V_T ventilation, there is no treatment that specifically targets either ARDS or respiratory failure in sepsis. The equivocal results of prior promising therapies are likely a function of the fact that both ARDS and sepsis represent heterogeneous populations. Kitsios and colleagues have demonstrated a means of parsing patients with these syndromes into previously unrecognized subgroups that may be more amenable to the development or recognition of effective therapies. Retrospective analysis of an earlier negative ARDS study comparing different positive end-expiratory pressure (PEEP) strategies suggest that a higher PEEP target may be beneficial if only applied to patients with ARDS with a hyperinflammatory subphenotype (7). Likewise, there is a possibility that such a tailored approach may also be needed in the treatment of sepsis. For example, two disparate therapies, steroids (an antiinflammatory agent) and immune checkpoint inhibitors (proinflammatory agents), have shown limited but not resolute success in the treatment of sepsis (8, 9). Both of these therapies could theoretically be beneficial (including within the same patient) depending on the patient's immune and microbiome profile and the timing of their clinical course. This approach of using the microbiome and/or the immune phenotype of a patient to guide treatment also has a role in the current efforts to identify targeted coronavirus disease (COVID-19) therapies, in which immunomodulating agents (mostly immunosuppressants; i.e., there are 10 active tocilizumab-based trials listed on clinicaltrials.gov) are being tested alongside therapies aimed at boosting the host's immune response (IFN-β-1a) to the virus (10–12). Finally, there may be a role for directly manipulating the host microbiome in patients with acute respiratory failure in a manner analogous to fecal microbial transplantation (FMT) for the treatment of *Clostridium difficile* or severe diarrhea in the setting of sepsis—both of which have been successfully treated in critically ill patients using FMT (2). In fact, it is conceivable that FMT may have a role in the management of acute respiratory failure, as it has been shown both in a murine model of sepsis and in patients with ARDS that the lung microbiome is enriched with gut microbiota (13).

In summary, the study by Kitsios and colleagues not only validates earlier work showing that a respiratory ecosystem that lacks microbial diversity and is dominated by host pathogens is associated with a poor clinical outcomes in patients with acute respiratory failure, but it also establishes a link between pulmonary dysbiosis and a hyperinflammatory state, which, in turn, is associated with worse outcomes in the critically ill, including ARDS. In the process, the authors have shown that acute respiratory failure, like many other critical illnesses, deserves more precise definitions if targeted therapies are to be identified. Indeed, future therapies for acute respiratory failure including ARDS may either be guided by dysbiosis patterns or may involve direct manipulation of the host's microbiome. ■

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References

1. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000;342:1334–1349.
2. Akrami K, Sweeney DA. The microbiome of the critically ill patient. *Curr Opin Crit Care* 2018;24:49–54.
3. Kitsios GD, Yang H, Yang L, Qin S, Fitch A, Wang X-H, *et al*. Respiratory tract dysbiosis is associated with worse outcomes in mechanically ventilated patients. *Am J Respir Crit Care Med* 2020;202:1666–1677.
4. Yeh A, Rogers MB, Firek B, Neal MD, Zuckerbraun BS, Morowitz MJ. Dysbiosis across multiple body sites in critically ill adult surgical patients. *Shock* 2016;46:649–654.
5. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, *et al*. Human gut microbiome viewed across age and geography. *Nature* 2012;486:222–227.
6. Kitsios GD, Yang L, Manatakis DV, Nouraie M, Evankovich J, Bain W, *et al*. Host-response subphenotypes offer prognostic enrichment in patients with or at risk for acute respiratory distress syndrome. *Crit Care Med* 2019;47:1724–1734.
7. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA; NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014;2:611–620.
8. Hotchkiss RS, Colston E, Yende S, Angus DC, Moldawer LL, Crouser ED, *et al*. Immune checkpoint inhibition in sepsis: a phase 1b randomized, placebo-controlled, single ascending dose study of antiprogrammed cell death-ligand 1 antibody (BMS-936559). *Crit Care Med* 2019;47:632–642.
9. Minneci PC, Deans KJ, Eichacker PQ, Natanson C. The effects of steroids during sepsis depend on dose and severity of illness: an updated meta-analysis. *Clin Microbiol Infect* 2009;15: 308–318.
10. National Institute of Allergy and Infectious Diseases (NIAID). Adaptive COVID-19 Treatment Trial 3 (ACTT-3). 2020. [accessed 2020 Aug 10] Available from: https://clinicaltrials.gov/ct2/show/NCT04492475.
11. Clinical trial to evaluate the effectiveness and safety of tocilizumab for treating patients with COVID-19 pneumonia. 2020 [accessed 2020 Aug 18]. Available from: https://clinicaltrials.gov/ct2/results?term=tocilizumab&cond=Covid19&Search=Apply&recrs=a&age_v=&gndr=&type=&rslt=.
12. Remy KE, Brakenridge SC, Francois B, Daix T, Deutschman CS, Monneret G, *et al*. Immunotherapies for COVID-19: lessons learned

from sepsis. *Lancet Respir Med* [online ahead of print] 28 Apr 2020; DOI: 10.1016/S2213-2600(20)30217-4.
13. Dickson RP, Singer BH, Newstead MW, Falkowski NR, Erb-Downward JR, Standiford TJ, *et al*. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat Microbiol* 2016;1:16113.

Turning the Lungs Inside Out: The Intersecting Microbiomes of the Lungs and the Built Environment

Ignore the protestations of our colleagues in gastroenterology: when it comes to surface area, the lungs beat the gut. While out-of-date textbooks claim that the gut lumen has a surface area comparable to that of a tennis court (260 m^2), some brave heretics recently used modern morphometric methods to show that prior estimates were greatly exaggerated (1). In the authors' words: "the total area of the human adult gut mucosa is not in the order of [a] tennis lawn, rather is that of half a badminton court": a modest 32 m^2 (Figure 1). In contrast, no one has dared challenge Philip Hasleton's 1972 estimate of the internal surface area of human lungs, 70 m^2, roughly that of a racquetball court (2). When it comes to surface area, the lungs take the prize at twice that of the gut and 30 times that of the skin. (Dermatologists have recently attempted to assert the supremacy of the skin by counting *intrafollicular* surface area (3), yet even this estimate is still only 25 m^2, merely one-third of a pickleball court.)

This vast surface area is also far more exposed to the outside environment than is the gut. To reach the lower gut lumen, ambitious microbes must traverse 6 m of bowel, enduring acidic and enzymatic assault, finally penetrating a thick, protective mucous layer. By contrast, no physical barrier (other than the intermittently closed larynx) separates the most distant alveoli from the outside environment, a mere half-meter away. Each day, the lungs are barraged by 7,000 L of air and all it contains. If your interest is the interface between the body and its environment, the lungs are where the action is.

It is fitting, then, that both sides of this interface—the lungs and the outside environment—have enjoyed recent revolutions in our microbiologic understanding. In the past decade, study of the lung microbiome has revealed that the lungs, long considered sterile, harbor diverse and dynamic communities of bacteria (4, 5), altered in disease (6–9), correlated with alveolar immunity (5, 10), predictive of clinical outcomes (6–8), and participating in pathogenesis (8, 11). Meanwhile, similar techniques interrogating "the microbiome of the built environment" have demonstrated that our residences, hospitals, and places of work have their own unique microbial communities. These environmental microbiomes are influenced by geography, season, outside air, building ventilation, and the animals and humans that inhabit the space (12, 13). The environmental microbiome contributes to the skin, nasal, and oral microbiota of its occupants (14, 15) and has been correlated with differences in health outcomes, including respiratory disease (16). Surprisingly, these fields have not yet intersected. We know next to nothing about whether and how the microbiota of our inhabited spaces influence the microbiota of our lungs.

In this issue of the *Journal*, Wu and colleagues (pp. 1678–1688) perform a remarkable double feat (17). First, they use microbiome methods to answer questions of pathogenesis in a rare occupational lung disease characterized by bronchiolitis, alveolar ductitis, and emphysema with B-cell primary lymphoid follicles (BADE) among patients exposed to metalworking fluids. Second, they provide the first evidence in humans that the microbiome of one's environment can seed and shape the lung microbiome.

The authors discovered that the lung microbiome in patients with BADE is distinct from that of matched BADE-free subjects, more closely resembling microbial communities detected in on-site metalworking fluid. To hone in on a microbial culprit, they used whole genome sequencing to show that BADE-associated bacteria in the lungs are genetically identical to *Pseudomonas pseudoalcaligenes*, a bacterial species known to dominate metalworking fluid, both in the literature (18) and in the facility in which the patients with BADE worked. Lastly, they established *in vitro* evidence of biologic plausibility by showing that B-cells (implicated in BADE) proliferate and are activated by in-use metalworking fluid. Together, these data link BADE not only to metalworking fluid but also the microbiota that inhabit it.

In addition to advancing our understanding of BADE, this study represents a step forward in our ecologic understanding of the human lung microbiome. Prior experimental work has demonstrated that manipulation of the environment can alter lung microbiota in mice and horses (10, 19), supporting our intuition that lung microbiota should reflect their outside environment. The current study takes the vital next step of testing this association in humans, using an impressive and exhaustive sampling strategy. To characterize the environment, they sampled microbiota from various types of metalworking fluid and air throughout the facility. Using these environmental taxa as a candidate "source community," they showed that the microbiome of the skin and nares of workers with close contact to metalworking fluid differed from those of more remote coworkers. Workers' physical proximity to metalworking fluid correlated with enrichment of the skin, nose, and lung microbiota with metalworking fluid–associated taxa.

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