How to Understand a Revolution: Guts, Lungs, and Bronchiectasis

Suppose a scholar seeks to understand the American Revolutionary War: its causes, consequences, and what lessons we may learn from it. They begin by reading *biographies* of key participants but soon realize that the scope of these books is too narrow. Broadening the scope to study *demography* (e.g., census reports, immigration records) helps them understand not merely the leaders of the revolution but also the people they led. However, even this isn't enough to understand the colonists' activities, so the scope is broadened further to *interactions*: in governance (political science), in the marketplace (economics), and in culture (sociology). Finally, our historian realizes that they cannot understand the events of Boston, Philadelphia, and Yorktown without also studying England: the leaders, people, and interactions that influenced a revolution from across an ocean.

This sequence—the broadening of one's scope of study to understand a complex historical phenomenon—provides us with a useful analogy for understanding another revolution: the introduction of culture-independent microbiology to our study of respiratory disease (1) (Table 1). For a century, the study of microbiota in the lungs relied on "biographies" of individual species: culture-based interrogation of prominent pathogens, studied by means of culture-dependent bacteriology and mycology. In the past decade, the use of microbiome techniques (amplicon sequencing, metagenomics) has revealed the complex "demography" of respiratory microbiota: diverse, dynamic populations of microbiota detected in healthy and diseased airways. However, with rare exceptions (2), the field has not reached the "interactions" scale of inquiry. We know little about the complex networks that exist between respiratory microbiota, the host, and mediators of disease pathogenesis. Furthermore, studies of the microbiome's role in lung disease have been anatomically compartmentalized, sampling either respiratory microbiota or gut microbiota, but never both. We've been trying to understand a revolution with an incomplete toolkit, looking through a narrow lens.

In this issue of the *Journal*, Narayana and colleagues (pp. 908–920) move the field forward by broadening our scope of inquiry. Seeking to better understand the microbiome's role in bronchiectasis, they studied a cohort of 57 adult patients with bronchiectasis (3). Although modest in size, the cohort was extensively and exhaustively characterized, both anatomically (both respiratory and gut specimens) and taxonomically (they studied both bacteria and fungi). This integrated strategy of cross-compartmental sampling and cross-kingdom sequencing is novel and enabled them to perform interactome analyses that incorporated both gut and lung communities, both bacterial and fungal.

Their core finding was that these patients with bronchiectasis cluster into two distinct subgroups, identified through their network analysis: a high gut-lung interaction group (dominated by lung *Pseudomonas*, gut *Bacteroides*, and gut *Saccharomyces*) and a low gut-lung interaction group (characterized by respiratory commensals such as *Prevotella* and gut *Candida*). Although these patient clusters were derived purely using microbiologic measurements, they differed clinically: The high gut-lung interaction cluster had worse clinical and radiographic severity as well as greater frequency of exacerbations. Although constrained by their observational study design (which precludes determining whether these clusters are a *cause* of disease severity or, instead, a *consequence* of it), the authors found that these clusters did not differ in exposure to chronic macrolides or inhaled corticosteroids, which suggests that they aren't mere artifacts of differences in treatment.

The most important lesson that we should draw from this work is the need to consider both respiratory and gut microbiota in human studies of respiratory disease. Although sputum microbiota have been studied extensively in bronchiectasis (4–6), and although gut communities have been implicated in the pathogenesis of other airway diseases (7, 8), the study by Narayana and colleagues is the first human study of any respiratory condition to integrate an analysis of microbiota across both anatomic compartments. Crucially, when the authors restricted their network analysis to gut-only and sputum-only communities, this diminished their ability to stratify patients into clinically distinct clusters. This key finding has important implications, both biological and methodological, for our understanding of the microbiome's role in respiratory conditions. Animal models that have sampled both compartments have demonstrated that the respiratory microbiome develops in tandem with that of the gut, and there is clear cross-talk between the two. Manipulation of gut microbiota can alter the microbiota and immunity of the lung, providing beneficial effects in asthma (9) and protection against respiratory viruses (10). Despite the compelling murine data of a "gut-lung axis," the human field has been siloed in parallel, nonintersecting inquiries: What is the role of gut bacteria in the calibration of immunity (both systemic and pulmonary) (11–13), and how is lung immune tone calibrated by local interactions with microbiota within the respiratory tract (14–19)? The study by Narayana and colleagues suggests that the correct answer to the question of "Which matters, gut microbiota or lung microbiota?" is "Both."

This study provides us with immediate, intermediate, and long-term implications. All prospective studies of the microbiome's role in respiratory disease should, if possible, obtain time-matched respiratory and lower gastrointestinal tract specimens. In the intermediate term, identification of exacerbation-prone patients (using gut and lung microbiota) may help risk-stratify patients and enrich clinical trials. This approach is already supported by a secondary analysis of the BLESS (Bronchiectasis and Low-Dose Erythromycin Study) trial—which demonstrated that clinical benefits of long-term erythromycin in bronchiectasis were specific to patients with *Pseudomonas*-enriched respiratory communities (6). In the long term, Narayana and colleagues provide new evidence supporting the potential targeting of both gut and respiratory microbiota as therapeutic targets in bronchiectasis (1).

Originally Published in Press as DOI: 10.1164/rccm.202211-2179ED on December 6, 2022

Table 1. Scales of Inquiry in the Study of History and Respiratory Microbiology

Scale of Inquiry	History	Microbiology
Individual agents	Biography	Bacteriology, mycology, virology
Populations	Demography	Microbial ecology (microbiome techniques)
Interactions within populations	Political science, economics, sociology	Network analysis
Local and remote influences	Study across countries (e.g., colonies and colonizers)	Study across anatomic compartments (e.g., lungs and gut)

This latter implication—that both gut and respiratory microbiota represent potential therapeutic targets in lung disease—forces us to confront the same challenge faced by our student of history: On which phenomenological scale do we focus our efforts? Will it suffice to eradicate specific species (such as *Pseudomonas*), either because they hold the potential to overgrow and cause infections (as pathogens) or because they play "keystone" roles within microbial networks? Or should we instead target collective features of microbial communities (such as bacterial burden and community diversity) (15, 16)? Or, instead, do we need to target networks and microbial interactions themselves, potentially by intercepting the mediators that propel and organize these bug-bug and bug-host interactions?

In reality, these domains of inquiry need each other: just as economics requires insights from demography and political science informs biography, we will need bacteriology, mycology, and microbial ecology to determine how these microbial networks assemble, dissolve, and participate in (or protect us from) disease pathogenesis. As valuable a tool as network analysis is, and as important as the findings of Narayana and colleagues are to the field, the answer to the question "At which scale do we target our interventions?" will surely be "all of the above." We have no more moved on from bacteriology, mycology, and microbial ecology than historians have moved on from biography and demography. If we seek to truly understand a revolution, we can't afford not to use all of our tools. ■

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

Callie M. Drohan, M.D.
Department of Internal Medicine
University of Michigan Health System
Ann Arbor, Michigan

Philip L. Molyneaux, M.B. B.S., Ph.D.
National Heart and Lung Institute
Imperial College London
London, United Kingdom

Robert P. Dickson, M.D.
Department of Internal Medicine
University of Michigan Health System
Ann Arbor, Michigan

Department of Microbiology and Immunology
University of Michigan Medical School
Ann Arbor, Michigan
and
Weil Institute for Critical Care Research & Innovation
Ann Arbor, Michigan

ORCID IDs: 0000-0003-2578-0413 (C.M.D.); 0000-0003-1301-8800 (P.L.M.); 0000-0002-6875-4277 (R.P.D.).

References

1. Chotirmall SH, Bogaert D, Chalmers JD, Cox MJ, Hansbro PM, Huang YJ, *et al.* Therapeutic targeting of the respiratory microbiome. *Am J Respir Crit Care Med* 2022;206:535–544.
2. Mac Aogáin M, Narayana JK, Tiew PY, Ali NABM, Yong VFL, Jaggi TK, *et al.* Integrative microbiomics in bronchiectasis exacerbations. *Nat Med* 2021;27:688–699.
3. Narayana JK, Aliberti S, Mac Aogáin M, Jaggi TK, Binte Mohamed Ali NA, Xaverius Ivan F, *et al.* Microbial dysregulation of the gut-lung axis in bronchiectasis. *Am J Respir Crit Care Med* 2023;207:908–920.
4. Cox MJ, Turek EM, Hennessy C, Mirza GK, James PL, Coleman M, *et al.* Longitudinal assessment of sputum microbiome by sequencing of the 16S rRNA gene in non-cystic fibrosis bronchiectasis patients. *PLoS One* 2017;12:e0170622.
5. Dicker AJ, Lonergan M, Keir HR, Smith AH, Pollock J, Finch S, *et al.* The sputum microbiome and clinical outcomes in patients with bronchiectasis: a prospective observational study. *Lancet Respir Med* 2021;9:885–896.
6. Rogers GB, Bruce KD, Martin ML, Burr LD, Serisier DJ. The effect of long-term macrolide treatment on respiratory microbiota composition in non-cystic fibrosis bronchiectasis: an analysis from the randomised, double-blind, placebo-controlled BLESS trial. *Lancet Respir Med* 2014; 2:988–996.
7. Stokholm J, Blaser MJ, Thorsen J, Rasmussen MA, Waage J, Vinding RK, *et al.* Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun* 2018;9:141.
8. Depner M, Taft DH, Kirjavainen PV, Kalanetra KM, Karvonen AM, Peschel S, *et al.*; PASTURE study group. Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nat Med* 2020;26:1766–1775.
9. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159–166.
10. Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J, *et al.* Dietary fiber confers protection against flu by shaping $Ly6c^{-}$ patrolling monocyte hematopoiesis and $CD8^{+}$ T cell metabolism. *Immunity* 2018;48:992–1005.e8.
11. Bongers KS, Chanderraj R, Woods RJ, McDonald RA, Adame MD, Falkowski NR, *et al.* The gut microbiome modulates body temperature both in sepsis and health. *Am J Respir Crit Care Med* [online ahead of print] 15 Nov 2022; DOI: 10.1164/rccm.202201-0161OC.
12. Chanderraj R, Baker JM, Kay SG, Brown CA, Hinkle KJ, Fergle DJ, *et al.* In critically ill patients, anti-anaerobic antibiotics increase risk of adverse clinical outcomes. *Eur Respir J* [online ahead of print] 13 Oct 2022; DOI: 10.1183/13993003.00910-2022.
13. Harris B, Morjaria SM, Littmann ER, Geyer AI, Stover DE, Barker JN, *et al.* Gut microbiota predict pulmonary infiltrates after allogeneic

hematopoietic cell transplantation. *Am J Respir Crit Care Med* 2016; 194:450–463.
14. Molyneaux PL, Mallia P, Cox MJ, Footitt J, Willis-Owen SA, Homola D, *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.
15. Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, *et al.* The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014;190: 906–913.
16. Dickson RP, Schultz MJ, van der Poll T, Schouten LR, Falkowski NR, Luth JE, *et al.*; Biomarker Analysis in Septic ICU Patients (BASIC) Consortium. Lung microbiota predict clinical outcomes in critically ill patients. *Am J Respir Crit Care Med* 2020;201:555–563.
17. O'Dwyer DN, Zhou X, Wilke CA, Xia M, Falkowski NR, Norman KC, *et al.* Lung dysbiosis, inflammation, and injury in hematopoietic cell transplantation. *Am J Respir Crit Care Med* 2018;198: 1312–1321.
18. 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.
19. 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: eaau9959.

Maldevelopment of the Immature Pulmonary Vasculature and Prolonged Patency of the Ductus Arteriosus: Association or Cause?

Nearly half to two-thirds of extremely preterm infants (<28 weeks of gestation) have moderate to large patent ductus arteriosus (PDA) shunts, which may lead to sustained exposure to excessive pulmonary blood flow (Qp) for days to weeks (1). Natural history studies of extremely preterm infants report a median time of PDA closure of 72 days (2). The association of PDA and bronchopulmonary dysplasia (BPD) is well documented; recent evidence highlights the increased likelihood of the most severe forms of BPD in patients with *both* prolonged mechanical ventilation and exposure to a large PDA (3) (Figure 1). The relationship of prolonged PDA exposure to pulmonary vascular maldevelopment, however, and its most severe form, pulmonary arterial hypertension (PAH), although biologically plausible, has received little scientific investigation. Almost 70 years ago, Hultgren and colleagues first reported the development of PAH, on either cardiac catheterization or autopsy, in eight patients after prolonged PDA exposure (4). Randomized clinical trials to date have focused exclusively on modulation of BPD, without any consideration of the impact on the immature pulmonary vascular bed or risk of PAH. Therefore, the direct effect of a prolonged PDA shunt, indirect effects through BPD development, *and* modulator effects of PDA treatment on pulmonary vascular remodeling and PAH remains unknown.

In this issue of the *Journal*, Gentle and colleagues (pp. 921–928) report a possible association between prolonged PDA exposure and PAH in a cohort of preterm infants, born at less than 28 weeks' gestational age, who remained on assisted respiratory support on Postnatal Day 28 (5). After adjustment for relevant covariates (birth weight, $F_{I_{O_2}}$ on Day 28), both PDA (adjusted odds ratio [aOR], 4.29; 95% confidence interval [CI], 1.89–9.77) and moderate to large PDA (aOR, 4.15; 95% CI, 1.78–9.64) were strongly associated with BPD–PAH at discharge. In addition, by probit analysis, each additional month of PDA exposure was associated with increased probability for the primary outcome of death or BPD–PAH at discharge. In an era when an increased number of clinicians have adopted a conservative approach to PDA, the implications at first glance may appear alarming. Although the findings are biologically plausible, several pathophysiologic factors need consideration before causality may be implied.

First, the interplay among pulmonary arterial pressure (PAP), Qp, and pulmonary vascular resistance (PVR) in neonatal PAH is explained by the equation PAP = PVR × Qp + left atrial pressure. Therefore, in the setting of prolonged PDA exposure, PAH may relate to developmental (pulmonary vascular remodeling and elevated PVR) or physiologic (excessive Qp and/or left atrial hypertension) factors (Figure 1). Although the latter is potentially modifiable with elimination of the ductal shunt, the former, which forms the basis of the authors' supposition, may be less amenable to pharmacological modulation. The relationship among oxygen exposure, target oxygen saturation, and ambient Pa_{O_2} is an additional, and poorly investigated, consideration in the setting of prolonged PDA shunt exposure. Intermittent or sustained periods of hypoxia, oftentimes followed by hyperoxia, may contribute to both pulmonary vascular injury and ongoing ductal patency (6). In addition, in the setting of a high-volume PDA shunt, oxygen saturation, which is a surrogate for hyperoxia exposure, is not a reliable determinant of oxygen free radical–mediated lung endothelial injury. Pulmonary overcirculation from left-to-right shunts, as observed with a large, unrestricted ventricular septal defect (VSD), is associated with excessive shear stress, leading to high risk of increased PVR and PAH (7). In addition, pulmonary arterial oxygen saturation (Spa_{O_2}) is increased in the setting of either PDA or VSD, thereby reducing the denominator in the shunt calculation: Qp/systemic blood flow = (Sa_{O_2} − mixed venous oxygen saturation)/(pulmonary venous oxygen saturation − Spa_{O_2}). Such high Spa_{O_2} may potentially enhance Qp and contribute to oxygen toxicity and endothelial dysfunction, resulting in PAH (7). It is therefore possible that high Spa_{O_2} and high pressure in infants with large PDAs might promote pulmonary vascular disease and PAH. Of importance, pressure- or stretch-induced vasoconstriction, known as the myogenic (or Bayliss)

Originally Published in Press as DOI: 10.1164/rccm.202211-2146ED on December 5, 2022