of CF, therapeutics that increase CFTR function to reduce and ultimately delay the onset and progression of CF lung disease appear to be within sight, providing great hope for people living with CF.

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The Challenging Road of Moving from Association to Causation for Microbiome Research in Idiopathic Pulmonary Fibrosis

In patients with idiopathic pulmonary fibrosis (IPF), respiratory infections are devastating events from which they often do not recover (1). Over the past decade, we have moved away from the use of immunosuppressive therapy and into an era of antifibrotic therapy. Although this has undoubtedly had a positive impact on the risk of acute infection in patients, the role of bacteria in the pathogenesis and progression of IPF does not end there. Over the past 5 years, there have been a number of studies highlighting the role of the bacterial communities (the microbiome) in the airways in IPF (2–5). Bacteria are persistent, present outside the context of an overt infection, and have been hypothesized to act as a continued driver of epithelial injury. A higher bacterial burden, disordered bacterial communities, and increased numbers of potentially pathogenic organisms have now all been associated with disease progression in IPF. The key word here, of course, is “association”: Despite elegant correlations with peripheral blood transcriptome signatures, inflammatory profiles, or genotypes, these studies have all boiled down to observations and correlations made in patients with this condition, albeit with plausible mechanisms underlying their conclusions. Causal inference requires many key elements that are frequently difficult to accomplish when studying human microbiota. Under the framework established by the Bradford Hill criteria, nine principles should be used to establish causation: strength, consistency, specificity, biological gradient, plausibility, coherence, analogy, temporality, and experiment (6). In this issue of the Journal, O’Dwyer and colleagues (pp. 1127–1138) try to tackle some of these remaining criteria through the use of a series of very elegant studies performed in preclinical models in an attempt to move us from association to causation (7).

The largest cohort study of the microbiome in IPF, published in the Journal in 2014, demonstrated that the overall bacterial load is
elevated in patients with IPF, and the higher the burden, the more rapid the disease progression (2). Using droplet digital PCR, a more sensitive and accurate measure than traditional quantitative PCR–based methods, ODwyer and colleagues set out to initially validate these findings. They were able to clearly demonstrate that subjects with progressive disease (based on a composite endpoint of clinically meaningful events) had a higher baseline bacterial burden than those with stable disease, just as in the initial study. Dichotomizing their cohort into tertiles of bacterial burden, the authors show striking differences in survival based on bacterial burden, with subjects in the highest group being five times at risk of disease progression compared with those in the lower tertiles, even when accounting for baseline disease severity. Interestingly, the authors were able to show differences in bacterial composition and diversity between these tertiles, with overall diversity reduced in the highest bacterial burden group. This drop in diversity, more than the bacterial burden, was associated with a proinflammatory and probiotic signal in the airways.

The authors therefore validate the finding that a higher bacterial burden at the time of diagnosis, in the absence of infection or immunosuppression, predicts progression in IPF. This finding now holds robustly true in independent cohorts using multiple quantification platforms, strengthening the observation. Yet, only two of the Bradford Hill criteria are met, consistency and biological gradient, and the observations remain associative rather than mechanistic. To address the issues of temporality and establish experimental conditions otherwise impossible in humans, the authors used a mouse model of fibrosis, which allowed them to explore the role of bacteria, through germ-free conditions, in lung inflammation and fibrogenesis. Although there are many issues and imperfections in any animal model of IPF, this work is a great example of how preclinical models can be used to interrogate specific questions raised from findings and observations made in relevant human cohorts (8). Before any germ-free mouse work, the authors needed to establish the impact of bleomycin on the respiratory microbiome in mice, which they set about doing using an oropharyngeal model. In this mouse model, there was no change in bacterial burden in lavage after exposure to bleomycin, but bacterial diversity increased rapidly during the inflammatory phase (0–7 d) in parallel with increases in alveolar protein. Switching to lung homogenate to study the fibrotic phase of the model, this dysbiosis clearly continued. Although there were a number of small changes in specific bacteria, the overall community structure of the respiratory microbiome in the mice remained grossly altered by bleomycin, and critically, these differences preceded the establishment of fibrosis.

Having established that bleomycin induces changes in the microbiome, the authors proceed to look at the effects of bleomycin in germ-free conditions. They found that the absence of a lung microbiome conferred a survival benefit despite the development of similar levels of fibrosis. The authors explain this apparent paradox by postulating that the altered microbiome may not be driving fibrosis directly but rather driving inflammation, a likely parallel pathophysiological mechanism. The authors found that germ-free mice had an increased number of regulatory T cells and reduced numbers of T-helper type 1 cells in their lungs. Murine data already exist to suggest that deregulated T-cell responses can exacerbate fibrosis (9, 10), although their role in human fibrotic lung disease remains unclear. Although the authors conclude that this survival benefit in the germ-free mice is likely driven by a decreased inflammatory tone, we should point out that the conditions in these experiments are not representative of adult human health. First, the extreme microbiota depletion of germ-free conditions is not comparable with any antimicrobial strategy in humans; second, these mice exhibit a profound immunological dysregulation because the microbiota is vital for normal host immune development. Thus, it is possible that some of the immunological signatures identified in the experiments performed might not be representative of conditions achievable in individuals with IPF. However, they provide a very important proof of concept that highlights a potential mechanism that will likely be followed by future investigations using gnotobiotic models (in which microbiota is reconstituted to mimic the human conditions) combined with immunologically targeted hosts achieved through knocking out specific mechanisms.

As in many other research areas, the challenge of moving from association to causation lies in repetition of human cohorts and preclinical models that set the stage for personalized therapeutic approaches that could then be tested in the setting of clinical trials. Although not achieving all nine Bradford Hill criteria, this work validates the finding that, in patients with IPF, BAL bacterial burden is able to predict survival and starts to bridge the knowledge gap between association and mechanism. Although future work must concentrate on elucidating these mechanisms further, we may now have a robust biomarker for disease progression in bacterial burden. Although prospective trials of broad-spectrum antibiotics in IPF are ongoing, the results of the study by ODwyer and colleagues suggest that a more nuanced or personalized approach maybe warranted that targets those patients with the highest burdens in whom the host immune tone in the lung is altered and may contribute to the overall prognosis and progression of disease.

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Despite the well-known detrimental health effects of cigarette smoking, rates of consumption remain high. Furthermore, rates of smoking are highest among women of childbearing age (20–24 yr), affecting one in six women. The risks of smoking during pregnancy include increased rates of miscarriage, prematurity, and low birth weight. Furthermore, the risks to the child from prenatal smoking extend well beyond the neonatal period and are known to include an increased risk of sudden infant death syndrome, low lung function, and lower respiratory tract infection (1). Despite these known risks, only one-quarter of women will quit prior to pregnancy and another 20% will quit during pregnancy (2), suggesting that many women are either not aware of the risks or are unable/unwilling to quit smoking.

The literature suggests that the prenatal period is a critical window for lung growth, which if altered has lifelong impacts. There is good evidence that prenatal cigarette smoking, and nicotine in particular, can directly impact the developing lung (1–5) and that the resultant adverse effects last a lifetime (6, 7). Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. Respir Res 2017;18:29.


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