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## 3 The Lung Microbiome in Health, Hypersensitivity Pneumonitis, and Idiopathic Pulmonary Fibrosis: A Heavy Bacterial Burden to Bear

The microbiome regulates human health and immunity, contributing robustly to physiological homeostasis. The healthy lung is not sterile and consists of bacterial communities that exist in a relatively low biomass state and correlate with local immunity (1, 2). In the diseased lung, there is a growing recognition of the potential mechanistic role of alterations or “dysbiosis” of lung microbiota (3). In particular, the lung microbiome has been implicated in the pathogenesis of idiopathic pulmonary fibrosis (IPF).

Studies in patients with IPF demonstrate that an increased burden of lung microbiota, as well as specific taxa such as *Streptococcus* and *Staphylococcus*, is associated with an elevated risk of disease progression and/or mortality (4–6). Lung microbiota are associated with innate immune activation signatures in peripheral blood (7), and increased  $\alpha$  diversity of lung microbiota correlates with lower alveolar inflammation (4). In animal models, lung dysbiosis precedes fibrosis and eradication of the microbiome significantly ameliorates fibrosis, suggesting a causal role for the lung microbiome, possibly through the activation of immune pathways (4, 8). Recently, Invernizzi and colleagues reported an

absence of correlations between key radiological markers and physiological features of IPF and lung bacterial burden, demonstrating that the increased bacterial burden reported in IPF is not simply the direct result of architectural distortion and parenchymal destruction (9). This addressed a key question in the field. However, it remains unknown whether these observations in IPF are universal to all interstitial lung disease (ILD).

Chronic hypersensitivity pneumonitis (CHP) is an enigmatic clinical syndrome and common form of ILD that frequently proves fatal. Both CHP and IPF share fibrotic remodeling of the lung parenchyma, may be indistinguishable by radiographic studies/histopathology, and respond to therapy directed at progressive fibrosis, supporting shared mechanistic pathways (10). Yet, patients with IPF fundamentally differ in prognosis (poorer in IPF, better in CHP), the presence of environmental antigen exposures (generally absent in IPF), and response to immunosuppression (detrimental in IPF, often beneficial in CHP), suggesting important differences between these diseases that are poorly understood.

In this issue of the *Journal*, Invernizzi and colleagues (pp. 339–347) report their findings on CHP and the lung microbiome in an elegant study (11). The authors compared key features of the healthy lung microbiome, the CHP microbiome, and the IPF lung microbiome. Patients with CHP exhibited a significantly lower lung bacterial burden compared with patients with IPF, although they still had greater lung bacterial burden compared with healthy subjects. However, bacterial burden was not associated with mortality in patients with CHP, unlike IPF, which is a fundamental new clinical observation. Furthermore, there were distinct differences in lung microbial composition between CHP

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and IPF. The lung microbiota of patients with IPF showed a greater abundance of *Firmicutes* and lower abundance of *Proteobacteria* compared with CHP. Interestingly, *Staphylococcus*, at the genus level, was more abundant in patients with CHP compared with IPF. However, the abundance of *Staphylococcus* was not associated with clinical outcomes in the CHP cohort, again unlike IPF. Overall, this paper supports the hypothesis that IPF pathogenesis is uniquely impacted by the microbiome and that the increased bacterial burden reported in IPF does not simply reflect the extent of underlying tissue fibrosis.

The paper by Invernizzi and colleagues has some noteworthy limitations. The study was performed as a single-center observational study and is limited in patient numbers. There are considerable differences in patient cohorts, with significant differences in age, sex, and disease severity at baseline. Although fibrosis is a commonality in CHP and IPF, the usual interstitial pneumonia pattern on radiographic studies predominated in IPF. There may be notable differences in community composition based on topography of the respiratory tract because previous studies have noted changes in bacterial communities based on the presence or absence of honeycombing in IPF (12). Furthermore, we lack an accurate understanding of the temporal changes that may occur in respiratory tract dysbiosis or the impact of immunosuppressive or antifibrotic therapy on the lung microbiome. The current study design with a single-time-point BAL cannot cater for this and important differences may be missed as a result. Because cellular and humoral immunological responses vary considerably between IPF and CHP, a more detailed analysis of immunological phenotype in the studied cohorts would have been revealing.

Nevertheless, this work exposes exciting new horizons. Recent reviews have highlighted the probable bidirectionality of host-microbiome interactions: an initial injury leads to dysbiosis, which in turn perpetuates injury (13). Perhaps patients with IPF may be uniquely susceptible to injury mediated by dysbiosis. In turn, lung bacterial communities in IPF may also be uniquely vulnerable to the impact of local physiological disturbances. It is remarkable that many IPF genetic risk factors involve innate immunity or host defense genes (14). In Invernizzi and colleagues' report, CHP mortality was <25% over 4 years as opposed to approximately 50% for IPF (11). Perhaps microbiome perturbations are not sufficient to impact CHP outcomes, but in IPF they suffice to perturb a fragile homeostasis enough to lead to morbidity and mortality. In this case, respiratory tract dysbiosis in IPF serves as a second hit/insult, which leads to decompensation.

Although these diseases share some common features, we must recognize that neither CHP nor IPF are monolithic disease entities but are likely to consist of several sub- or endotypes. For example, genetic risk factors that are associated with IPF have also been identified in CHP (15), and certain patients with CHP exhibit the radiological features and adverse outcomes of IPF (16). This suggests that subgroups of CHP may share pathomechanisms, prognosis, and perhaps lung dysbiosis characteristics with subgroups of IPF, and as such, future work may require more detailed clinical, immunological, and microbiological endotyping.

In conclusion, this paper reports a fundamental observation for the field of lung microbiome science and ILD. An increased bacterial burden in the respiratory tract of patients with CHP is not associated with mortality, whereas in patients with IPF, the increased bacterial burden elevates the risk of death. For patients with IPF, this

increased bacterial load is indeed a heavy burden to bear. An improved understanding of this key observation will require further mechanistic work and carefully designed longitudinally observational studies. This future research will further advance us toward better treatments and, hopefully, a cure for these devastating diseases. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

David N. O'Dwyer, M.B. B.Ch., B.A.O., Ph.D.  
University of Michigan Medical School  
Ann Arbor, Michigan

Stavros Garantziotis, M.D.  
National Institute of Environmental Health Sciences  
Bethesda, Maryland

ORCID ID: 0000-0003-4007-375X (S.G.).

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## Branching Out in Chronic Cough: Evidence for Increased Airway Nerve Density

Chronic cough is believed to affect up to 12% of the general population and most commonly occurs as a consequence of cigarette smoking and chronic respiratory diseases (e.g., chronic obstructive pulmonary disease, asthma, and bronchiectasis) (1, 2). In patients presenting to specialist cough clinics, the cause is often less obvious, and the cough is more likely to be associated with nasal disease, gastroesophageal reflux disease, or asthma. In some patients, the chronic cough may not improve with the treatment of these conditions and is termed “refractory” or if no cause can be identified, “unexplained.” Patients attending cough clinics mainly describe a dry/minimally productive cough triggered by trivial exposures to innocuous stimuli such as changes in temperature, environmental irritants (e.g., perfumes and cleaning products) and use of their voice (3). Coughing occurs typically hundreds of times per day and has significant quality of life impacts, especially if treatment of associated conditions is not beneficial. Currently there are no licensed therapies for chronic cough, and the underlying mechanisms are poorly understood. In recent years, it has become increasingly accepted that a hyperexcitability of the neuronal pathways controlling cough is likely to be a fundamental component of the pathophysiology. Indeed, heightened cough reflex responses have been demonstrated in patients with chronic cough in studies experimentally evoking cough (4). However, the precise nature of this hyperexcitability remains unclear, and the extent to which this may reflect changes in activation/function of the airway nerves responsible for initiating cough and/or their connections in the central nervous system is unknown.

In this issue of the *Journal*, the study by Shapiro and colleagues (pp. 348–355) examined whether airway nerve density is increased in patients with chronic cough (5). Remarkably few studies have visualized airway nerves in bronchoscopic biopsies in respiratory disease, probably as a consequence of the methodological challenges. Achieving adequate staining of neuronal elements is difficult in human tissue, perhaps because of inadequate

penetration of the immunohistochemical stains or the lack of specificity of antibodies raised against neuronal targets in other species. Thus, visualization of neurons is mainly achieved with the panneuronal stain PGP9.5, and the presence of other receptors to further characterize the innervation is limited (6). Moreover, once neuronal staining patterns are achieved, describing the three-dimensional neuronal architecture in an objective standard manner is problematic.

Authors of this study are to be congratulated on developing a technique for generating three-dimensional models to facilitate the quantification of nerve length and branching as a means to describe nerve density (7). Using this technique, they found that a group of 22 patients presenting with chronic cough (more than 12 wk duration) had increased epithelial nerve density (both increased length and branching) compared with a group of well-matched healthy volunteers. Subepithelial nerve density was no different between the groups, and there were also no differences in staining with substance *P* or eosinophil peroxidase. This intriguing finding raises the possibility that increased epithelial innervation may play a role in the pathophysiology of chronic cough. The study confirms the previous finding of nonsignificant increases in epithelial nerve density in patients with chronic cough (8), adding detail about neuronal length and branch points indicative of neuroplasticity.

The factors mediating neuronal branching are complex and vary considerably with different types of neurons (9). Most is known about mechanisms in the brain, where the primary function of branching is to form new synaptic connections. However, increased branching/sprouting of sensory neurons also occurs in peripheral tissues, including in lesional skin in atopic dermatitis (10) and colonic mucosa of irritable bowel disease (11). Animal models of cystitis/overactive bladder, painful arthritic joints, and breast cancer-induced bone pain have also described increases in nerve density (12–14). Branching is stimulated by extracellular factors, such as guidance molecules, neurotrophic factors, and adhesive ligands, and by intracellular organelle position and gene expression (9). Branching can be activity dependent but may also occur as a compensatory mechanism after neuronal damage. In the study by Shapiro and colleagues, the increased density of epithelial fibers may therefore have occurred as a consequence of many processes; these include not only the direct effects of the shearing forces and pressures generated by coughing but also the resultant release of inflammatory mediators (e.g., ATP). Based on the location and morphological features of the fibers studied, they are most likely to be vagal C fibers, which are predominantly sensitive

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