Lung Microbiomes: New Frontiers?

Most studies of the human microbiome have provided snapshots of the bacterial diversity in the lung and gastrointestinal tract (1–5). However, fungi play a large role in human health and disease and represent an important, understudied taxa. In this issue of the *Journal*, Cui and colleagues (pp. 932–942) report the results of a study to characterize the fungal microbiome, termed the "mycobiome," of the respiratory tract (6). They explored the wide gaps in our understanding of chronic obstructive pulmonary disease (COPD), the potential role of colonizing fungi in COPD, and how mycobiomes are altered in the presence of HIV. The investigators performed an observational study of the lung mycobiome at one point in time for 56 participants who were recruited from San Francisco and Los Angeles in California and from Pittsburgh, Pennsylvania, excluding persons with other chronic comorbidities and those who were currently using antibiotics. In multivariable models, they adjusted for smoking history as a potential confounder of the associations between fungal pathogens and COPD in HIV-infected individuals.

The investigators used novel, culture-independent tools to investigate the fungal mycobiome of the oral and respiratory tract. They extracted fungal genomic DNA from oral wash, induced sputum (IS), and bronchoalveolar lavage (BAL) samples and bronchoscopic controls from each participant. By polymerase chain reaction, they amplified the 18S and internal transcribed spacer of the rRNA gene and sequenced the 18S and internal transcribed spacer amplicons. Nested polymerase chain reaction was used to confirm the presence of fungal species in BAL samples. Using the genomic sequences, the investigators identified the fungal species present in oral wash, IS, and BAL samples from each participant. Such novel, culture-independent tools have the potential to be incorporated into the clinical microbiology laboratories of the future if further studies prove that microbial communities in the respiratory tract cause poor patient outcomes and that interventions that alter these microbial communities are beneficial to patients.

Interestingly, the investigators report that the mycobiomes of oral wash, IS, and BAL shared common organisms, but also had species that were disproportionately prevalent. For example, *Pneumocystis jirovecii* was significantly more prevalent in the fungal communities in BAL among participants with HIV and COPD. The greatest number of fungal species (n = 225) was detected in the oral wash, although *Candida* species were, as expected, the most common. The fungal mycobiome in IS specimens had little in common with the BAL specimens. The investigators detected significant differences in the fungal communities by HIV status and COPD. The study also presents preliminary data suggesting that participants who smoked marijuana during the previous year were more likely to have additional fungal species in the lung. The lack of a filter with most forms of marijuana use predisposes patients to colonization and infection with a number of plant pathogens, including highly pathogenic organisms such as *Aspergillus* species (7). The effect of marijuana smoking on lung health and disease remains poorly understood and merits further investigation.

There are important limitations from this study that need to be addressed as future studies are designed. Patient recruitment for clinically unnecessary procedures was undoubtedly difficult, thereby limiting sample size and patient matching and imposing a selection bias. Twenty-four adults without HIV infection were included as the control group, but sampling is unlikely to detect informative inter- or intraperson variability and to represent the population of persons with a "normal" lung mycobiome. Nor can we assume that the 32 participants with HIV infection are representative of HIV-infected individuals with different viral loads and CD4 counts. Previous studies, for example, demonstrated that viral loads were more strongly associated with the presence of *Candida* species among people with HIV infection (8, 9). In addition, samples were collected at a single time at different locales and under different ecologic conditions (e.g., location, temperature, and humidity), factors that can have a large effect on patients' exposures and fungal dispersion or transmission, growth, and pathogenesis. The immunosuppressive effect of corticosteroids (systemic or inhaled), which are widely used in the treatment of COPD (10), was not adequately addressed in the present study. The heterogeneous microbial habitats within the lung, which may favor the growth of certain species over others (11), were not examined or used as criteria in the decision to obtain BAL samples. No data were presented comparing the relative sensitivity, specificity, and positive and negative predictive values of the 18S and internal transcribed spacer methods versus cultures for the fungal species. As acknowledged by the authors, the participants' self-reported information about smoking and marijuana use might be affected by recall bias. There is no information describing what the fungi were doing in the oral cavity and lung, or even if they were alive. Importantly, the study does not provide any data that demonstrates an association between a fungal species or multiple species and clinical outcomes. The clinical relevance of their findings is therefore limited.

Nevertheless, the article by Cui and colleagues (6) is thought provoking and suggests many new questions about the mycobiome of the respiratory tract. For example, do the bacterial and viral species in a microbiome interact with and affect the growth, pathogenesis, and transmission of fungal species? Recent studies suggest that *Pseudomonas aeruginosa* and fungi inhibit each other's growth in the respiratory tract, but we do not know whether this is a common phenomenon between different taxa or different species in the same taxa (12). Do certain microenvironmental conditions in the lung, such as temperature, humidity, CO_2, and O_2 concentrations, also determine the mycobiome that forms and flourishes? What defines the normal, healthy human mycobiome in the respiratory tract (13, 14)? Can detectable, significant disruptions or changes in one's mycobiome over time be used as markers of worsening lung health? Can the mycobiome in the oral cavity and lung be altered and used as empiric therapy or prophylaxis (15)? The study by Cui and colleagues (6) is an important step toward the use of novel tools and multidisciplinary

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approaches for well-designed prospective cohort studies of the natural history of mycobiomes, as well as clinical trials to test the effect of specific microbial communities, antibiotics, antivirals, and other treatments and interventions. ■

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A Reader's Guide to the Bactericidal Activity of Pyrazinamide and Clofazimine Alone and in Combinations with Pretomanid and Bedaquiline

In this issue of the *Journal*, Diacon and colleagues (pp. 943–953) assessed the 14-day extended early bactericidal activity (EBA) of pyrazinamide alone, clofazimine alone, and four combination regimens of bedaquiline in permutations with pyrazinamide, pretomanid (Pa824), and clofazimine (1). The positive treatment control was a standard regimen of rifampin, isoniazid, pyrazinamide, and ethambutol, delivered as a combination tablet (Rifafour; sanofi-aventis, Paris, France), and the study participants were treatment-naive patients with pulmonary tuberculosis with positive results from sputum-smear microscopy for acid-fast bacilli. On Day 14 of treatment, the pharmacokinetic (PK) parameters of the prescribed drugs alone and in combination were measured.

The results were clear-cut. First, each of the three experimental three-drug combinations (bedaquiline, pretomanid, and pyrazinamide; bedaquiline, pretomanid, and clofazimine; and bedaquiline, pyrazinamide, and clofazimine) and the one experimental four-drug combination (bedaquiline, pretomanid, pyrazinamide, and clofazimine) had extended EBA that was not significantly different from that of the Rifafour control regimen. Second, pyrazinamide alone had minimal EBA, as expected, and clofazimine alone provided no early activity at all. Third, the main PK parameters of each studied drug were apparently not affected by the other drugs given in combination; that is, drug–drug interactions were not apparent, and PK parameters were mostly within the expected range. For clofazimine, the peak plasma concentration after 14 days of daily administration was, on average, 0.2 μg/ml and close to its 0.25 μg/ml minimum inhibitory concentration for *Mycobacterium tuberculosis*. The peak plasma concentrations and 24-hour area under the concentration curve of bedaquiline, its M2 metabolite, and clofazimine varied six- to eightfold across regimens and among individuals, but the median exposures achieved were relatively similar across regimens; there was less variation in pretomanid and pyrazinamide exposures.

Such results are informative. Despite bedaquiline's ability to shorten the time to culture conversion in patients with