Exploring the Parallel Development of Microbial Systems in Neonates with Cystic Fibrosis

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ABSTRACT Recent studies have greatly extended our understanding of the microbiota present in and on the human body. Here, advanced sequencing strategies have provided unprecedented analytical power. The important implications that the emerging data have for human health emphasize the need to intensify research in this area (D. A. Relman, Nature 486:194-195, 2012). It is already clear from these studies that the microbiotas characterized in different body locations of healthy individuals are both complex and diverse (The Human Microbiome Project Consortium, Nature 486:215-221). These studies also provide a point of contrast for investigations that aim to characterize the microbiota present in disease conditions. In this regard, Madan et al. (mBio 3(4):e00251-12, 2012) monitored the development over time of microbiota in the oropharynges and feces of neonates with cystic fibrosis and explored the potential for interactions between these complex microbial systems.

Since its first full description some 70 years ago, cystic fibrosis has been the focus of considerable clinical and scientific research. We now know cystic fibrosis to be a common genetic disease, in which the mutation underpinning CF results in altered ion transport. While this impacts multiple body systems, most clinical concern arises over the airways and the chronic infections that typically develop there. Through these infections and the resulting host response, lung damage occurs that is key to morbidity and mortality in this patient group. Understanding the microbes that drive these airways infections is therefore important. A decade ago, diagnostic microbiology was focused on the culture-based detection of a small set of bacterial and fungal species that were considered of clinical significance. From 2003 onward, however, a series of studies showed through culture-independent analysis that a complex mix of bacteria (1), fungi (2, 3), and viruses (4) form the airway microbiota of an individual chronically infected with CF. Recent studies on the chronically infected airways have shown associations between the bacterial microbiota of sputum and a range of clinical parameters over time (5, 6). A continued research effort is now needed to better understand how these data can inform the process of improving the health of CF patients (7). The airways are not the only body system affected in CF. Abnormal mucus production in the small intestine is thought to contribute to gastrointestinal (GI) symptoms of malabsorption and obstruction. Evidence that the fecal microbiota of CF patients differs from those of individuals without CF also exists (8). Combining these points, it is possible that altered microbiotas may have important negative effects on the growth, development, and physiology of a child with CF.

To date, then, work in this field has focused primarily on the airway microbiota and most typically on that in patients that are chronically colonized. This is, in part at least, due to the need to better understand the microbiota and the impact of antibiotic therapies that have been key to improving well-being and extending life. This emphasis on the airway microbiotas of chronically infected CF patients, however, misses important questions on how the microbiotas in the airways and gut assemble. Is this a stochastic process, or do founding members determine the microbiota composition at these and other body sites in later life? Does this explain differences in health outcomes, and could this lead to directed interventions to "steer" the microbiota in directions beneficial to the host? To start to answer these questions, we need to look at CF at earlier stages. A number of studies have reported on the airway microbiota of young individuals with CF (9–11). In their study, Madan et al. (12) looked still further back by examining neonates with CF, something that is itself enabled by diagnostic advances in next-generation high-throughput sequencing technologies, and tracked patients through the first 21 months of life.

In that paper, "gut" and "respiratory" were used to describe the samples taken. While this is fair, some further clarification may be helpful. Microbiota present in fecal samples were used to represent the gut. Although this is a commonly used strategy, studies have shown that the composition of fecal microbiota differs from that of the microbiota associated with biopsy samples (13). Madan et al. also used oropharyngeal swabbing to characterize the airway microbiota. Here, a more direct sampling of the lung itself would be more helpful, but as the authors comment, the processes needed to sample the lower airways, e.g., bronchoalveolar lavage, are invasive. As the authors also discuss, there is evidence to suggest that there is a correlation between the species present in the oropharynx and the lower airways. The exact relationship is, however, not clear, and this is an area that needs more detailed research. With these caveats in mind, so as to avoid confusion, the terms "gut" and "respiratory" are used in this commentary. It should be stressed, though, that the collection of clinical samples that the team assembled is unique and represents a strength of the work. The culture-independent 16S rRNA gene 454 pyrosequencing method used to study the bacterial genera that were present over time was similar to that employed in previous studies. Here, though, it is essential to highlight the importance of statistical methodologies, such as intraclass correlation coefficient analysis and recursive partitioned mixed modeling, as tools for interpreting this class of data. The combination of such tools with pyrosequencing approaches is another marked strength of the work.

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So, what was found? The first observation was that overall, a relatively limited set of eight genera was common, including *Streptococcus*, *Prevotella*, and *Veillonella* in respiratory samples and *Bacteroides*, *Bifidobacterium*, and *Veillonella* in gut samples. The presence of the genera *Veillonella* and *Prevotella* in respiratory samples is consistent with findings that report the same anaerobic genera in many chronically colonized CF patients. This identification of key groups of bacteria here presents an opportunity to make comparisons with those reported in analogous studies outside this clinical context. For example, comparisons with studies focusing on the respiratory or gastrointestinal tracts of non-CF individuals could allow the extent to which microbiota composition reflects the underlying physiological changes that are associated with this condition to be determined. The authors also determined that interindividual consistency was greater for respiratory than for gut microbiotas. Overall, too, while this study found that microbiotas fell into two clusters—one comprising respiratory samples and the other gut samples—this small group of genera was common to both. This concept of identifying core, as opposed to transient, microbiota membership within a metacommunity has been used before in a study of CF (14).

Reflecting the unique longitudinal nature of the data set, some of the most interesting findings came through a comparison of the genus-level data over time. By tracking the temporal dynamics of the microbiota, the authors identified a significant increase in bacterial diversity over time, with the fastest increase being detected among the respiratory samples. Not only is this of potential importance, as the authors state, in relation to disease severity (15), but there may also be implications for immunodevelopment, as set out in the biodiversity hypothesis (16).

In the study by Madan et al. (12), the same genera that were seen to increase in the gut were also found to increase in respiratory samples. Also, certain genera were found to be present early in the neonatal gut and then later in the respiratory samples as well; i.e., "gut colonization presages their appearance in the respiratory tract." Again, the authors were able to show significant links for the cluster patterns of bacterial genera in respiratory samples, such as associations with breastfeeding and the introduction of solid foods into the diet of the child.

With regard to study limitations, the caveats set out above emphasize the need to exercise caution when interpreting the presence of species as being "definingly" gut or respiratory in nature. It might be interesting to reconsider aspects of the findings in relation to the means of sampling; oropharyngeal swabs contact the epithelial surface directly, whereas fecal samples are by their nature a (variable) mix of food and cellular contributions of a variety of sources. As the authors state, clear limitations also include the number of CF patients monitored and the frequency of sample collection. At this point, however, it is important to credit the authors for their ability to involve and maintain the commitment to the study of the parents in the program.

The contributions of this study to future directions of work in this field are likely to be seen as particularly important. The authors propose interventional studies in terms of diet or probiotics as attractive longer-term options. While the debate that this will generate is in itself welcome, there is much to be done to first reinforce and then extend what is presented in this paper. CF clinicians are concerned about the acquisition of certain species, such as *Pseudomonas aeruginosa*, which are associated with a range of poor clinical outcomes. Studies of why key species such as this colonize CF patients at different ages are warranted—is this linked to the composition of the microbiota at time of colonization? As for studies in later CF, it is also important to consider other components of the microbiota in parallel through, for example, metagenomics-based analysis. Microbiota studies need to be tied more specifically to clinical outcome, with concepts of mechanism being needed as much as intervention. Clearly, then, there is a need for a set of long-term studies that link microbiota with clinical outcome.

To some extent, the importance of this study is in its initiation of work in this area, with more questions emerging than perhaps might have been hypothesized originally. It is important to reflect that for many years following the full description of the condition in the late 1930s, this study would not have been possible—life expectancy then was still 6 months on average. While the advances made in CF should not be underestimated, we need to continue to move forward. Microbiota studies provide tremendous opportunities to gain new insights and understanding. This needs support in order to develop, but the promise of clinical benefit that microbiota studies will allow is great. The paper by Madan et al. initiates activity in microbiota research in neonatal CF and is to be commended as such.

REFERENCES

1. **Rogers GB, et al.** 2003. Bacterial diversity in cases of lung infection in cystic fibrosis patients: 16S ribosomal DNA (rDNA) length heterogeneity PCR and 16S rDNA terminal restriction fragment length polymorphism profiling. J. Clin. Microbiol. **41**:3548–3558.
2. **Bouchara JP, et al.** 2009. Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. J. Clin. Microbiol. **47**:142–152.
3. **Delhaes L, et al.** 2012. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community—implications for therapeutic management. PLoS One **7**:e36313.
4. **Willner D, et al.** 2012. Case studies of the spatial heterogeneity of DNA viruses in the cystic fibrosis lung. Am. J. Respir. Cell Mol. Biol. **46**: 127–131.
5. **Stressmann FA, et al.** 2012. Long-term cultivation-independent microbial diversity analysis demonstrates that bacterial communities infecting the adult cystic fibrosis lung show stability and resilience. Thorax **67**: 867–873.
6. **Zhao J, et al.** 2012. Decade-long bacterial community dynamics in cystic fibrosis airways. Proc. Natl. Acad. Sci. U. S. A. **109**:5809–5814.
7. **Han MK, et al.** 2012. Significance of the microbiome in obstructive lung disease. Thorax **67**:456–463.
8. **Duytschaever G, et al.** 2011. Cross-sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. Appl. Environ. Microbiol. **77**:8015–8024.
9. **Harris JK, et al.** 2007. Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. Proc. Natl. Acad. Sci. U. S. A. **104**:20529–20533.
10. **Armougom F, et al.** 2009. Microbial diversity in the sputum of a cystic fibrosis patient studied with 16S rDNA pyrosequencing. Eur. J. Clin. Microbiol. Infect. Dis. **28**:1151–1154.
11. **Cox MJ, et al.** 2010. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One **5**:e11044.
12. **Madan JC, et al.** 2012. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. mBio **3**:e00251-12.
13. **Eckburg PB, et al.** 2005. Diversity of the human intestinal microbial flora. Science **308**:1635–1638.
14. **van der Gast CJ, et al.** 2011. Partitioning core and satellite taxa from within cystic fibrosis lung bacterial communities. ISME J. **5**:780–791.
15. **Vandevanter DR, Lipuma JJ.** 2012. Microbial diversity in the cystic fibrosis airways: where is thy sting? Future Microbiol. **7**:801–803.
16. **Hanski I, et al.** 2012. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. U. S. A. **109**:8334–8339.