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MINIREVIEW

Cystic Fibrosis Airway Microbiome: Overturning the Old, Opening the Way for the New

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ABSTRACT The genetic disease cystic fibrosis (CF) is associated with chronic airway infections that are a proximal cause of death in many patients with this affliction. Classic microbiology studies focusing on canonical pathogens resulted in the development of a common set of views regarding the nature of the airway infections associated with this disease, and these ideas have influenced everything from the way infections are treated to how clinical trials for new CF-targeted antibiotics are designed and the focus of CF-related research topics. Recent culture-independent studies have prompted us to rethink, and in some cases discard, some of these long-held views. In this piece, I argue that an updated view of the complicated chronic infections associated with CF, thanks in large part to culture-independent studies of sputum and bronchoalveolar lavage fluid samples, should be leveraged to develop new strategies to treat these recalcitrant infections.

KEYWORDS cystic fibrosis, microbiome, infection, sputum, airway

Mary Anderson first described "cystic fibrosis of the pancreas," now known as cystic fibrosis (CF), in 1938 (1), and it was recognized fairly early on that airway infection is a significant component of the CF disease process (2). As children lived longer and grew into adulthood, the progression of CF-associated airway disease became, and remains, a major clinical problem. Thus, the efforts to understand the microbiology of infections in the CF airway intensified (3). Classical culture-based clinical microbiology approaches for the identification of pathogens defined some key players in these chronic airway infections and simultaneously helped develop commonly held views regarding which microbes caused lung damage and how the microbial signature of infection changes over the course of infection (3, 4).

In more recent years, arguably starting in 2004 with a report by Rogers et al. (5), culture-independent approaches have been used to try to gain an additional understanding of the CF airway microbiota. This work has not been without controversy. In particular, questions often arise as to whether the sputum used as a source of DNA for culture-independent studies might be contaminated with oral microbiota and, importantly, what the extent of this contamination is, thus misleading the interpretation of the findings (6–9). A second critique often voiced is whether sputum is truly representative of the airway microbiota more broadly (7). Of course, these issues are also relevant to classical microbiology analysis of sputum, with the caveat that plate-based studies often employ a selective medium that supports the growth of only a portion of the microbes in the sample (3, 10). Nevertheless, the question has been raised for microbiome studies as to whether a single sputum sample truly represents the breadth of microbes present in the CF airway.

I would argue that lost in this critical narrative are a number of important contributions from these culture-independent studies to our thinking about the composition and role of microbes in the airway infections that are critical components of CF disease

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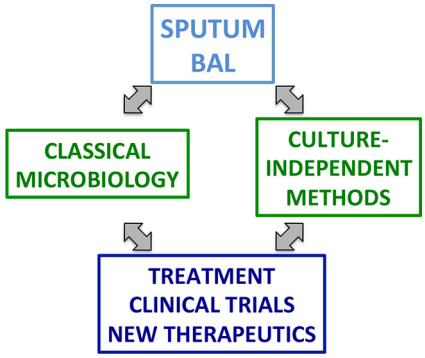

FIG 1 Role of culture-independent approaches for studying the microbiology of the CF airway. Sputum and BAL fluid, in most cases, are the sources of material used for elucidating the microbiology of the CF airway. New culture-independent methods have built on and complemented classical microbiology approaches. The culture-independent methods (largely 16S rRNA gene sequencing) have provided new insight into the nature of these infections.

(Fig. 1). Indeed, as presented here, several "dogmatic" views of these chronic infections, often restated in the introductions of papers or just simply "general knowledge," have been overturned by recent culture-independent studies. Importantly, the rethinking of our understanding of CF-associated airway infections on the basis of this new evidence positions us to deal with our current reality. While antibiotic therapies and the new waves of CF transmembrane conductance regulator (CFTR) corrector and potentiator drugs have the potential to mitigate airway infection, many patients still bear the burden of long-term lung function decline due to chronic infection (11). Reconsidering our long-held views of CF microbiology should also help the community of CF researchers and clinicians to rethink how to treat these chronic infections.

I have decided to take a somewhat unusual tack in the format of this review article. I first present the "classic" view of CF microbiology developed from culture studies and clinical observations and then highlight how that view must be altered in light of culture-independent work. It is important to note that our current consensus understanding cannot often be pinpointed to a particular publication; rather, these views are commonly held and recited when the field discusses issues related to microbiology and infection in CF.

"*PSEUDOMONAS AERUGINOSA* IS THE DOMINANT BUG IN >80% OF CF PATIENTS"

Many talks or papers discussing CF microbiology typically include some variation of the statement, "The airway of >80% of CF patients is dominated by *P. aeruginosa*," including work from my own lab (see references 12 to 21 for some examples). This statement is likely derived from plots of culture data showing that up to 70 to 80% of adult CF patients are culture positive for this important CF pathogen (22). It is also important to note that the terms "dominant" and "prevalent" have been sometimes conflated in the literature. As outlined below, I define dominant as meaning the majority of microbes in the infection are *P. aeruginosa*. Prevalent, in contrast, is defined as a microbe found among a large number of patients. Thus, a microbe can be prevalent (i.e., found in 90% of patients) but not dominant (for example, only comprising a small percentage of the microbes present). As outlined below, we see that while *P. aeruginosa* is indeed prevalent, it is the most abundant microbe and thus dominant in only ~40% of CF patients. Thus, as illustrated below, the statement "The airway of >80% of CF patients is dominated by *P. aeruginosa*" is not formally correct.

A number of recent culture-independent studies of adults and children with CF dispute the view that *P. aeruginosa* is the dominant pathogen in most patients with CF if we define dominant colonization by *P. aeruginosa* as >50% of the total reads from culture-independent analysis of the 16S rRNA gene. My review of 16 different studies that include 507 patients (9, 12, 15, 18, 23–35) shows that only 42.4% of the airways of

adult CF patients are dominated by *P. aeruginosa*. As an aside, it is important to note that the abundance and prevalence of *P. aeruginosa* vary with the age of the patient, with a general reduction in diversity of the airway communities in older patients and examples of total dominance of the CF airway by *P. aeruginosa* in end-stage disease as determined by analyzing tissue samples of such lungs removed during transplant (12, 32, 36–39). As outlined above, however, the picture of end-stage disease appears to be distinct from that of patients who still retain higher levels of lung function. Additionally, a recent study by Limoli et al. analyzing >200 adult patients with CF showed that *P. aeruginosa* was detectable in approximately one-third of the patients, while one-third had a mixture of *P. aeruginosa* and *S. aureus*, and the remaining third were culture positive for neither organism (40). Such findings are more than simply academic observations, given that the study design for currently approved antibiotics targeting pathogens in CF, as well as the treatment regimens for these patients, including eradication therapy, are largely focused on targeting *P. aeruginosa* (41–55). Such information may also be useful for clinicians making the often complicated decision about what antibiotic(s) should be used to treat patients with these complex infections. Similarly, clinical trial designs may need to incorporate a baseline microbiome study and potentially stratify patients by the dominant microbe present in the individual. If such baseline and endpoint microbiome studies are adopted, it is also critical to recognize that the lack of dynamics in culture-independent studies may reflect, at least in part, the stability of DNA (versus viable microbes) in sputum (56). The focus on the reduction in *P. aeruginosa* burden as an endpoint for the success of a trial for a new antibiotic may effectively ignore the microbiota and/or pathogens of over half of the patients with CF or even alter the "success" or "failure" outcomes of such studies. Interestingly, studies of the efficacy of aztreonam for inhalation solution (AZLI), an antibiotic that targets *P. aeruginosa* in the context of the CF airway, used alternative outcomes of benefits to CF patients, given the modest impact of this antibiotic on *P. aeruginosa* burden (15, 20, 48, 57). It may be that the benefit observed for AZLI arose from the impact on microbes other than *P. aeruginosa*. Given recent findings of the broad diversity of microbes in CF patients, it may be reasonable to consider other clinical trial outcome metrics.

P. AERUGINOSA BURDEN INCREASES WITH EXACERBATION AND IS REDUCED WHEN THE EXACERBATION IS TREATED WITH ANTIBIOTICS

A common refrain in explaining the cause of exacerbation was an increase in either *P. aeruginosa* numbers or bacterial load more broadly (58, 59). Patients diagnosed with an exacerbation (admittedly, a poorly defined condition) are then hospitalized and treated with intravenous (i.v.) antibiotics (58–61). The i.v. antibiotic chosen is typically informed by the antibiotic resistance profile of the *P. aeruginosa* isolates cultured during the last quarterly visit to the clinic.

Recent microbiome studies tell a different story. Using a common study design of recruiting stable CF patients and then tracking these patients during the course of exacerbation, the treatment and subsequent exacerbation resolution revealed no significant relationship between absolute or relative levels of *P. aeruginosa* or the total load of bacteria (measured by 16S rRNA gene) and the onset or resolution of the exacerbation (13, 18, 32, 62–65). Thus, these 7 studies of 102 patients revealed no obvious or consistent pattern in community structure, *P. aeruginosa* abundance, or overall bacterial load that could explain the onset of exacerbation. In contrast, a study from several years ago by Sibley et al. (65), combining culture and culture-independent approaches to dissect airway communities in CF, as well as follow-up studies from this and other groups (12, 23, 24, 27, 66–68), suggests the underappreciated role of *Streptococcus* spp. in CF airway disease, including the link of the *Streptococcus milleri* group (*S. constellatus*, *S. intermedius*, and *S. anginosus*) to at least a subset of exacerbation events. The work of Surette's group provides what is perhaps the most compelling mechanism for exacerbation onset, but a role for the *S. milleri* group microbes could only account for ~40% of exacerbations. The impact of culture-independent

microbiome studies, alone or combined with culture, on the thinking about exacerbations is reflected in a 2016 review article by Smyth (58) highlighting the need to rethink exacerbation diagnosis and treatment in light of these recent data. As it stands, while the older view of factors driving exacerbation appears to be off base, we do not yet understand what triggers an exacerbation event.

SPUTUM DOES NOT REFLECT THE MICROBIOLOGY OF THE AIRWAY

A review of the literature relevant to the microbiome and the CF airway would not be complete without a discussion of whether sputum acts as a relevant surrogate for the microbiota in the CF airway. This discussion has disproportionately dominated the narrative regarding the value of such microbiome studies. While end-stage disease, represented by the analysis of transplanted lungs for example (6, 36, 37, 69), is indeed commonly associated with *P. aeruginosa*-dominated infections, several studies have independently verified that the lung microbiota in CF is polymicrobial in nature and well reflected in sputum samples, at least for the major genera identified. A few recent studies speak to this controversy.

Rogers et al. (70) used 16S rRNA gene profiling by terminal restriction fragment length polymorphism analysis to compare mouthwash and sputum samples from 19 adults with CF. Based on this analysis, they concluded that their sputum samples were not subject to "profound contamination by oral cavity bacteria" (70). They based their conclusion on the markedly different profiles and the lack of overlap between mouthwash and sputum samples. That is, if oral microbes were substantially contaminating sputum, then the two samples should look quite similar—this is not what these investigators observed (70). Using a complementary approach, Hogan et al. (9) compared the profiles of microbes from spontaneously expectorated sputum samples with those from bronchoalveolar lavage (BAL) fluid and/or protected brush samples. Based on the analysis of 6 patients for which there were both sputum and bronchoscope-harvested samples, they concluded that, for typical CF pathogens (*P. aeruginosa*, *Stenotrophomonas*, *Achromobacter*, *Burkholderia*, *Staphylococcus*, and *Haemophilus* spp.), there was a concordance between the dominant pathogen from sputum and BAL fluid samples by deep sequencing and classical microbiological approaches (9). Protected brush sampling of the airway, using a methodology that shielded the sample from oral contamination, revealed that *Streptococcus*, *Rothia*, *Peptostreptococcus*, and *Gemella* are indeed found in the airway and cannot be explained by oral contamination. It is important to note that while this study did show clear differences between sputum and BAL fluid, likely driven in part from the contribution of oral contaminants, sputum used for culture-independent or classic microbiological analysis provides important information regarding the microbiota of the CF airway.

Another important point raised by the study from Hogan et al. (9) comes from regional (upper, middle, and lower) sampling of the right airway by BAL or protected brushes. These investigators found no significant difference among the communities between regions or as a function of differential regional lung damage (9). These data suggest that the original source of the sputum may not matter in terms of the information provided. Also consistent with this finding is a previous study of serial sputum samples from adults showing consistent communities over a 7-month period (71). Similarly, a recent study of the microbiota from 95 daily sputum samples of four patients with CF showed little in the way of day-to-day changes (72). Taken together, these data can be taken to mean the source of the sputum in the airway does not dictate the microbes identified; rather, the relative uniformity of the microbial community in the airway means that sputum samples are quite likely to paint the general picture of the microbiota present in the lung. Furthermore, given the complexity of standard quantitative microbiological counts from clinical samples, the relative low cost of culture-independent methods, and the advantage of determining relative and absolute abundance values of microbes in the airway, sputum analysis could provide an important aspect of the overall clinical picture.

Finally, the controversy regarding oral contamination of sputum has also potentially

resulted in undervaluing the role of anaerobes in CF airway disease. Publications from several groups, including the works of Gilligan and of Tunney and colleagues, using a combination of culture-dependent and -independent studies to analyze sputum and bronchoalveolar lavage fluid samples have associated anaerobes and their metabolic products with negative respiratory outcomes and inflammation and demonstrated that these microbes can be nonresponsive to typical CF-specific antimicrobial treatments (5, 9, 73–78). Thus, the narrative of oral contamination of sputum should not close the door on exploring the potentially important impact of anaerobes in CF airway infection.

CONCLUSIONS

The value of microbiome studies in the context of the CF airway, in this author's opinion, has been underestimated. A discussion dominated by methodological debates and driven by considerations that appear to stem from misleading paper titles has resulted in many outside the field underappreciating CF airway microbiome studies over the past decade. In fact, data from the culture-independent analysis of sputum and bronchoalveolar lavage fluid samples should (and can) be impacting everything from the therapeutic choices of physicians to clinical trial design. I would argue that culture-independent studies of sputum and bronchoalveolar lavage fluid from the CF airway have overturned several long-held, but inaccurate, ideas in the field. The enhanced, and clearly more complex, picture of the nature of infections in the CF airway will enable the field to move forward. While antibiotic therapies have benefited many CF patients, there are clearly limitations of the current antimicrobial therapeutic regimens. Perhaps our newly enhanced understanding of the important role of pathogens other than *P. aeruginosa*, as well as the reality of polymicrobial biofilm-like infections in the CF airway, will spur new thinking, model systems, and strategies to treat these chronic infections.

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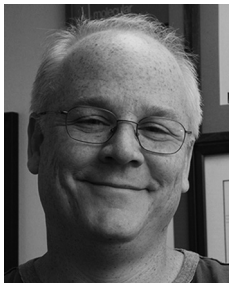

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