Good cop, bad cop: anaerobes in cystic fibrosis airways

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The role(s) that anaerobic bacterial species play in the complex microbial communities inhabiting the airways of people with cystic fibrosis has been the subject of several recent investigations. These studies have demonstrated seemingly conflicting results. http://ow.ly/Qom630kB9DG

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For much of the 80 years since the initial descriptions of cystic fibrosis (CF), the microbiology of CF airway infection focused on a small set of aerobic bacteria known to be capable of causing human infection. Although anaerobic bacteria could be recovered from CF sputum [1], their presence was most often attributed to contamination by anaerobes in the upper airway. Further, the oxygen-rich environment of the respiratory tract was thought to provide a less than favourable niche to sustain appreciable numbers of strictly anaerobic bacteria. Work by Worlitzsch *et al.* [2] some 15 years ago showed that steep oxygen gradients occur in mucus in CF airways. Subsequent research by others, including recent work in Dianne Newman's laboratory at Cal Tech, has corroborated these findings, providing compelling evidence that intraluminal conditions in CF airways are capable of supporting the growth of both facultative and obligate anaerobic bacteria [3]. These studies have been complemented during the same 15-year period by research utilising culture-independent (*i.e.* DNA sequence-based) approaches to characterise CF airway microbial communities. These analyses have consistently shown that anaerobic bacteria are both highly prevalent and present in considerable abundances in CF airways [4–9].

The debate about whether anaerobic bacteria are or are not a significant component of CF airway microbial communities is thus giving way to growing interest in better defining the role anaerobic species play in CF airway infection [10]. Several recent studies have addressed this by seeking associations between the presence of anaerobes and clinical outcomes in CF or by exploring mechanistic hypotheses about how anaerobic species may impact lung disease progression. These studies have produced seemingly contradictory results with respect to the question of whether anaerobes contribute to lung pathology or if they may, in contrast, somehow ameliorate disease progression. Experimental evidence suggests, for example, that anaerobic bacteria can elicit strong proinflammatory responses *in vivo* [11]. Work by Mirković *et al.* [12] and by Ghorbani *et al.* [13] has shown that short-chain fatty acids produced by anaerobic bacteria through fermentation mediate the release of proinflammatory cytokines from human bronchial epithelial cells *in vitro*, an effect that is more pronounced in CF cells than in normal bronchial epithelium. The positive correlation between short-chain fatty acid levels and neutrophils observed in CF sputum suggests a mechanism whereby anaerobes enhance neutrophil recruitment into CF lungs *in vivo* [13].

Other recent work has highlighted interactions between anaerobes and conventional (aerobic) CF pathogens that may contribute to the pathogenicity of the latter. For example, work in Ryan Hunter's laboratory has shown that anaerobic species commonly found in CF respiratory specimens (*Prevotella*,

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Veillonella, Fusobacterium and anaerobic streptococci) have the ability to degrade respiratory mucins to produce amino acids and short-chain fatty acids that may serve as nutrient sources for, and enhance the growth of, conventional CF pathogens, including *Pseudomonas aeruginosa* [14]. Work by several groups has shown that 2,3-butanedione, a fermentation product of several bacterial species, including anaerobic streptococci, increases *P. aeruginosa* pyocyanin production and biofilm formation *in vitro* and promotes *P. aeruginosa*-induced inflammation in a murine airway infection model [15–17]. Still other research has shown that extended-spectrum β-lactamases produced by some anaerobes may protect *P. aeruginosa* and other CF pathogens from the activity of β-lactam antimicrobials [18].

While these studies provide mechanistic and pathophysiologic bases for the role of anaerobes in contributing to CF lung disease, other lines of investigation seem to suggest that the presence of anaerobes may have a positive effect on preserving lung health. In cross-sectional analyses, Zemanick *et al.* [7, 19, 20] found that sputum and bronchoalveolar lavage samples with higher relative abundance of anaerobes were associated with lower inflammation and higher forced expiratory volume in 1 s compared to samples with higher relative abundance of *P. aeruginosa* or *Staphylococcus*. In a longitudinal study of children receiving ivacaftor, Bernarde *et al.* [21] similarly observed a positive correlation between the relative abundance of certain anaerobic species and lung function. Work in George O'Toole's laboratory found that increased relative abundance of facultatively anaerobic streptococci was the strongest predictor of clinically stable lung disease [22]. A limitation of these studies is their reliance on estimates of species relative abundances, wherein a decrease in the relative abundance of some species must be reflected in the increase in the relative abundance of others. This limitation was overcome in a study by O'Neill *et al.* [23], who used quantitative bacterial culture to show that lower levels of viable anaerobic bacteria were associated with worse lung function and increased inflammation.

Thus, while experimental data have identified potential mechanisms whereby anaerobes may promote CF lung disease progression, either alone or in concert with other bacterial species, observational analyses characterising the structure of CF airway microbial communities appear to suggest a beneficial role for anaerobes. What are we to do with these seemingly discordant observations? A practical, clinically relevant question is should we more specifically target anaerobes with antimicrobial therapy, or would strategies to better preserve anaerobes in diverse airway microbial communities yield better clinical outcomes?

In this issue of the *European Respiratory Journal*, Muhlebach *et al.* [24] provide additional analyses aimed at better understanding the role anaerobic species play in CF airway infection. By applying extended bacterial culture methods to sputum and bronchoalveolar lavage specimens from a large age range of people with CF, these investigators identified age-related prevalence rates of anaerobic species and described relationships between anaerobes and clinical outcomes. For the sake of this study, the authors defined anaerobes as including only obligate, or strict, anaerobic species; facultative anaerobes, including streptococci, or species that can grow anaerobically in oxygen-limited conditions, were categorised with aerobic species. Nevertheless, consistent with prior studies, the presence and abundance of anaerobes were positively associated with markers of milder CF disease, including better lung function, body mass index and pancreatic sufficiency.

The use of bacterial culture in this study complements previous studies employing culture-independent analyses to profile airway bacterial communities. While the use of culture is not without caveats (*e.g.* bacteria are not evenly dispersed in sputum, and culture media and conditions impact results), this approach circumvents limitations inherent in DNA sequence-based analysis, including distinguishing viable from non-viable bacteria, variable target gene copy number, and differential lysis of bacterial cells. And as above, the use of quantitative culture allows an estimation of absolute species density, compared to the measures of species relative abundance provided by DNA sequence-based community profiling. Finally, bacterial culture allows for species-level identification of bacteria, a degree of taxonomic resolution that is often not possible with bacterial 16S rRNA gene sequencing. Although most of the analyses performed by the investigators used genus-level categorisation, the detailing of bacterial species (both aerobic and anaerobic) recovered in this large sample set provides an outstanding resource with which to better assess species-level epidemiology, which, in turn, has potential to inform future mechanistic studies.

Based on finding a positive association between anaerobes and milder lung disease, the authors conclude that antibiotic therapy targeting anaerobes may not be warranted in managing CF airway infection. However, the study's design limits broad conclusions in this regard. The study is primarily a cross-sectional analysis of anaerobe prevalence in sputum samples taken from individuals during periods of baseline health. Previous experimental and observational studies of airway microbiota around the time of pulmonary exacerbations paint a different picture of the potential role for anaerobes in CF. Quinn *et al.* [25] used an *in vitro* system to simulate CF airways and demonstrated an increase in anaerobe abundance and fermentative metabolism during pulmonary exacerbations. A recent cross-sectional analysis of several

hundred CF sputum samples using culture-independent bacterial profiling similarly showed an increase in the relative abundance of anaerobic species at the onset of exacerbation, particularly in subjects with early or intermediate lung disease [9]. These data suggest a role for anaerobes in pulmonary exacerbation and provide rationale for considering the inclusion of antimicrobial agents with activity against anaerobes in the management of exacerbations, perhaps depending on patients' lung disease stage. Clearly, further study is needed before definitive, and more refined, recommendations can be made regarding antimicrobial targeting of anaerobes in CF.

The authors were careful not to overreach in drawing conclusions regarding causality in describing the association between anaerobe prevalence and milder lung disease. As they acknowledge, previous studies have consistently shown a positive correlation between CF airway bacterial community diversity and milder lung disease [26]. A corollary observation has been that the reduction in community diversity associated with increasing patient age and lung disease reflects the progressive domination of the community by a conventional CF pathogen (*i.e. P. aeruginosa*, *Burkholderia* spp. or *Achromobacter* spp.), which occurs at the expense of other species, including anaerobes. The authors' data again demonstrate this; the prevalence rates of obligate anaerobes and *Streptococcus* decreased during the first two decades of life, while the prevalence of *Pseudomonas* steadily increased. Of interest, following this decline, the prevalence rates of anaerobes and *Streptococcus* were found to increase in individuals older than 25 years. This inflection point in anaerobe prevalence rates corresponds to the current median age of death in CF, suggesting a "survivor effect"; older individuals with higher rates of anaerobes (*i.e.* greater microbial community diversity) are likely to have a milder lung disease phenotype.

Further investigation of the intriguing relationships between airway microbial diversity, anaerobe abundance and lung function in CF is needed. Does the prevalence and/or abundance of anaerobes merely reflect the lack of domination by a conventional CF pathogen, which seems to portend late stage disease? Is the presence of anaerobes a reflection of other factors (*e.g.* inflammation, antibiotic use) that more directly impact lung health? Conversely, do anaerobes, or perhaps just certain anaerobic species, play an active role in mitigating CF lung disease (*e.g.* through antagonism of more pathogenic species or inhibition of inflammation)? Addressing these questions to untangle the causal relationships underlying the observations described by Muhlebach *et al.* [24] presents a challenge. But advancing our understanding of the role of anaerobes in CF, with studies such as this, promises to pay dividends in improved management of CF airway infection.

Conflict of interest: None declared.

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