



The Yin and Yang of *Streptococcus* Lung Infections in Cystic Fibrosis: a Model for Studying Polymicrobial Interactions

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ABSTRACT The streptococci are increasingly recognized as a core component of the cystic fibrosis (CF) lung microbiome, yet the role that they play in CF lung disease is unclear. The presence of the *Streptococcus milleri* group (SMG; also known as the anginosus group streptococci [AGS]) correlates with exacerbation when these microbes are the predominant species in the lung. In contrast, microbiome studies have indicated that an increased relative abundance of streptococci in the lung, including members of the oral microflora, correlates with impacts on lung disease less severe than those caused by other CF-associated microflora, indicating a complex role for this genus in the context of CF. Recent findings suggest that streptococci in the CF lung microenvironment may influence the growth and/or virulence of other CF pathogens, as evidenced by increased virulence factor production by *Pseudomonas aeruginosa* when grown in coculture with oral streptococci. Conversely, the presence of *P. aeruginosa* can enhance the growth of streptococci, including members of the SMG, a phenomenon that could be exacerbated by the fact that streptococci are not susceptible to some of the frontline antibiotics used to treat *P. aeruginosa* infections. Collectively, these studies indicate the necessity for further investigation into the role of streptococci in the CF airway to determine how these microbes, alone or via interactions with other CF-associated pathogens, might influence CF lung disease, for better or for worse. We also propose that the interactions of streptococci with other CF pathogens is an ideal model to study clinically relevant microbial interactions.

KEYWORDS *Streptococcus*, *Streptococcus milleri* group, cystic fibrosis, exacerbation, polymicrobial

CYSTIC FIBROSIS LUNG INFECTIONS ARE POLYMICROBIAL AND INCLUDE STREPTOCOCCI

Patients with cystic fibrosis (CF) have a thick, dehydrated mucus in their airway, which reduces mucociliary clearance and allows for colonization by bacteria. The lung infections in CF patients have been demonstrated to be polymicrobial and complex (1–3), and correlations between certain lung pathogens and declining lung function have been established, as demonstrated for *Pseudomonas aeruginosa* (4), despite the current high level of antibiotic treatment and eradication strategies (5). Historically, *Streptococcus* spp. isolated from airway-derived sputum samples were considered to be oropharyngeal contaminants from the process of expectoration (6) or were not isolated at all in clinical laboratories (7). However, streptococci are increasingly recognized as members of the CF lung microbiome, having been cultured from and identified through 16S rRNA gene sequencing of bacteria from literally thousands of sputum and lavage samples (1, 2, 6–21). Streptococci have also been found in multiple protected brush samples collected using a bronchoscope (11); using such protected brushes greatly reduces the risk of contamination by oral flora and supports the conclusion that these microbes are indeed found in the lower airway in patients with CF. As outlined below, specialized medium is required for selecting for the growth of streptococci (7,

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22); thus, these organisms can often be missed during routine plating of clinical samples from patients with CF (20). Finally, given the proximity of the oral cavity and the lower airway, it is not surprising to find in the lung microbes that have been seeded from the oral microbiota (23–28). Thus, the presence of *Streptococcus* in the CF airway available to interact with *P. aeruginosa* and other organisms should not come as a surprise.

Here, we explore the complex role of streptococci in CF-associated airway infections (Fig. 1), in particular, the observation that streptococci, in a species-dependent fashion, either may be associated with a lower disease burden than typical CF pathogens (2, 9, 12, 21, 29) or, alternatively, may contribute to pulmonary exacerbations (1, 6, 17–19). We also review recent studies indicating that streptococci influence and can be influenced by other microbes in the context of a polymicrobial infection in patients with CF.

THE *STREPTOCOCCUS MILLERI* GROUP AS AN AGENT OF EXACERBATION

Several studies have characterized the correlation between the *Streptococcus milleri* group (SMG; also known as the anginosus group streptococci [AGS]) and pulmonary exacerbation (1, 7, 17, 30). These studies demonstrate that when SMG isolates are the numerically dominant pathogens in the lung, patients experience an exacerbation that will not resolve until the levels of SMG in the airway have been adequately reduced through antibiotic therapy (1, 7, 17, 30). Utilizing a semiselective agar medium for the detection of SMG isolates, Sibley and colleagues found that these microbes have an overall prevalence of ~40% in their patient population and that in cases where the patient was hospitalized for a pulmonary exacerbation, SMG isolates were observed to reach numerical dominance ($>10^7$ CFU per milliliter of sputum) (7). Another group demonstrated that nalidixic acid and sulfamethazine (NAS) agar also allowed for semiselective quantification of SMG isolates from sputum samples; these investigators isolated members of the SMG from 6 out of 10 sputum samples from patients with CF (31). Together, these data suggest that the members of the SMG represent significant (and underappreciated) pathogens in CF airway disease. Furthermore, it was demonstrated that in 73% and 29% of cases, respectively, *P. aeruginosa* and *Staphylococcus aureus* were found to cocolonize patients with SMG isolates, indicating that this group of streptococci may interact with these better-known CF pathogens, which could affect the virulence and growth of these pathogens (7).

IS THERE A ROLE FOR TYPICAL PATHOGENIC STREPTOCOCCI IN CF?

As discussed above, streptococci found in the CF airway are typically associated with the normal oral flora. However, recent studies suggest that pathogenic streptococci that are typically associated with acute infections of the respiratory tract can also be found in the airways of patients with CF. For example, 15 of 318 (4.7%) adults examined in a retrospective study were culture positive for group A streptococcus (GAS) in their sputum, and 7/15 of these patients were suffering from an exacerbation at the time of the positive culture (32), analogous to the observations made for SMG isolates and exacerbation described above. In another study, ~20% of the 212 children with CF studied were shown to have detectable *Streptococcus pneumoniae* when oropharyngeal swab specimens were analyzed by quantitative PCR (33). Interestingly, Dennis and colleagues showed that mucoid variants of *S. pneumoniae* could be isolated from the sputum of children with CF (32). Furthermore, while these mucoid strains showed reduced initial biofilm formation *in vitro* compared to nonmucoid strains, they eventually formed more robust biofilms and were more virulent in a mouse model of CF. Thus, the phenotypes of the mucoid and nonmucoid *S. pneumoniae* strains mirror those of mucoid and nonmucoid *P. aeruginosa* strains (34, 35). The mucoid phenotype of the *S. pneumoniae* isolates was due to capsule production, and these strains switched to a nonmucoid phenotype, likely via phase variation, while growing in biofilms (32, 36), which is likely their mode of growth in the context of a CF-related infection.

The interaction of *P. aeruginosa* with *S. pneumoniae* has not been investigated in any

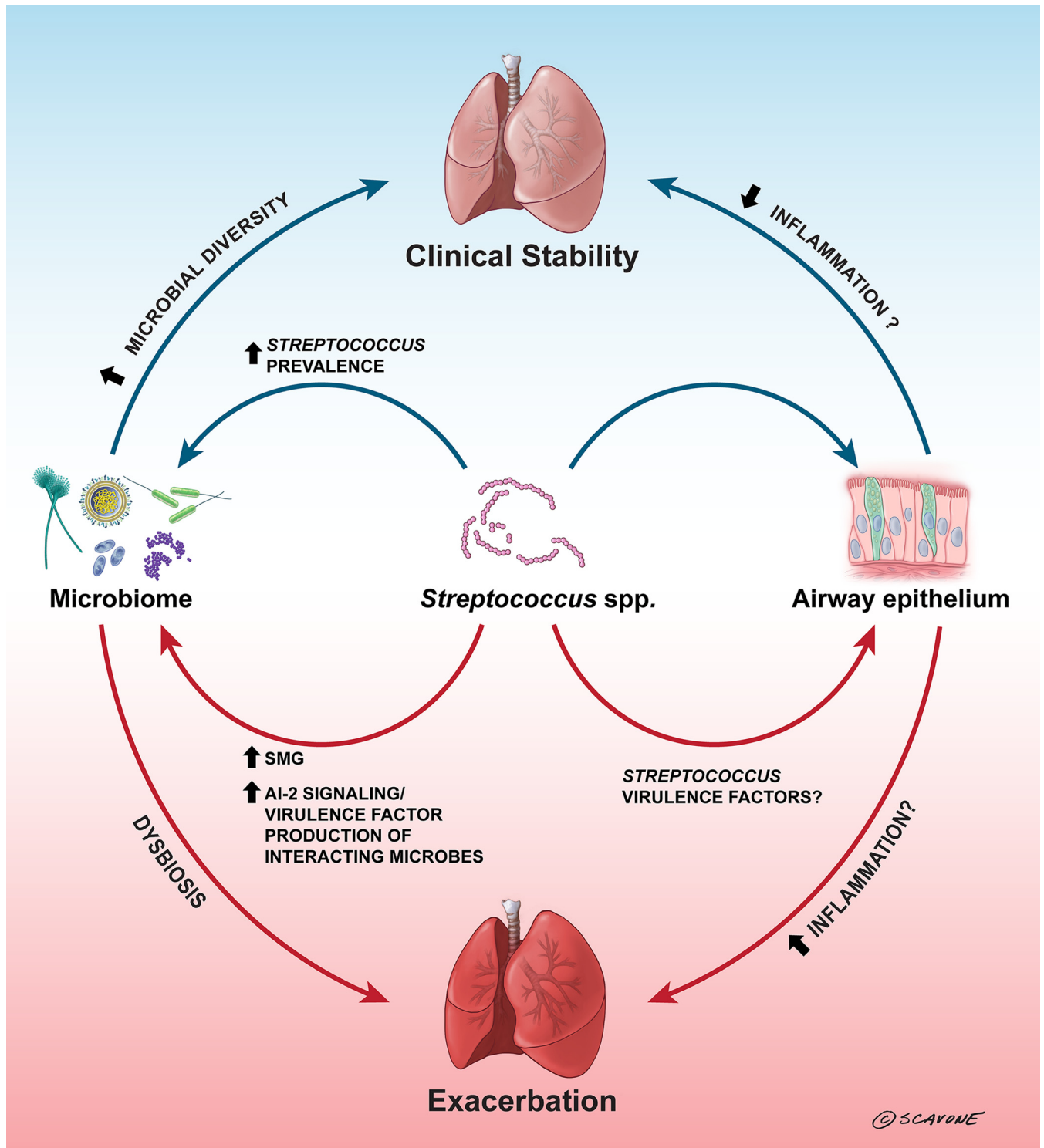


FIG 1 Streptococci can influence cystic fibrosis airway disease. In CF airways, an increased prevalence of *Streptococcus* spp. has been correlated with clinical stability, lower inflammation, and/or less severe outcomes (2, 9, 12, 21, 29), but it is unclear whether this is due to direct interactions with the airway epithelium, is due to interactions between the streptococci and the microbiome that lead to increased microbial diversity, or is a consequence of less damage to the airway. Oral streptococci have been demonstrated to inhibit *P. aeruginosa* growth through the production of hydrogen peroxide and reactive nitrogenous intermediates (49–51); by inhibiting *P. aeruginosa*, streptococci could open up niches for increased microbial diversity in the lung, which could aid clinical stability. In contrast, the predominance of SMG isolates has been correlated with exacerbation (1, 7, 17, 30), and this may be due to interactions between the SMG isolates and other CF pathogens, such as *P. aeruginosa*. Oral streptococci have been demonstrated to stimulate increased virulence factor production by *P. aeruginosa* (43–45) through AI-2 signaling (43), and this could lead to dysbiosis in the lung and eventual exacerbation. The arrows indicate both direct and indirect interactions that are suggested to occur in the CF airways. The blue arrows indicate generally positive effects, the red arrows indicate generally negative effects, and the arrows are labeled with the factors (if known) involved in these effects. (Copyright William Scavone, Kestrel Studio; reproduced with permission.)

depth, but one report suggests a mechanism whereby *P. aeruginosa* could promote the growth or persistence of *S. pneumoniae*. The LasB elastase, whose production can be stimulated by streptococci, as discussed below, was shown using *in vitro* studies to reduce the efficacy of alveolar macrophages in the clearing of both *P. aeruginosa* and *S. pneumoniae* by ~2-fold (37). These data indicated that *P. aeruginosa* could modify the airway environment to promote the persistence of *S. pneumoniae*. Therefore, we would suggest that future studies of *P. aeruginosa*-*Streptococcus* interactions include *S. pneumoniae* and GAS, as well as the more typical CF-associated streptococcal species.

STREPTOCOCCI ARE ASSOCIATED WITH DISEASE LESS SEVERE THAN THAT CAUSED BY TRADITIONAL CF PATHOGENS

Five recent microbiome studies indicate that colonization of the CF lung by oral *Streptococcus* spp. may be associated with a disease burden less severe than that associated with typical CF pathogens (2, 9, 12, 21, 29). Work from our group has indicated that an increased relative abundance of members of the genus *Streptococcus* in the lung, determined by 16S rRNA gene sequencing, correlates with clinical stability (2). Another study utilizing 16S rRNA gene sequencing indicated that the relative abundance of streptococci was significantly lower in patients with CF in the lowest quartile of forced expiratory volume in 1 s (FEV₁), a key marker of lung function (9); thus, a low relative abundance of streptococci (and, thus, a higher abundance of other CF pathogens) was associated with poorer lung function. It has also been demonstrated that the relative abundance of streptococci increases when patients undergo CF transmembrane conductance regulator modulation therapy with ivacaftor, while the relative abundance of *P. aeruginosa* declines (12). In another study, Acosta et al., studying a cohort of 104 patients with CF (18 to 22 years of age), showed that higher levels of *Streptococcus* were associated with a decreased likelihood of progressing to early end-stage lung disease (29). Furthermore, Coburn et al., examining 269 patients over a 60-year age range, identified *Streptococcus* spp. to be part of the core lung microbiota (as assessed via analysis of sputum) and observed a greater degree of dominance of this microbe in younger patients (<25 years of age) and in patients with less severe disease (21). The same study (21) observed that when *Streptococcus* spp. were the dominant organisms, the overall diversity of the community was higher in both pediatric and adult patients. This finding is consistent with that of a longitudinal study of CF patients reported by Zhao and colleagues, especially for the samples from early in life (38). Finally, a separate study suggests that the increased diversity in CF airway communities is beneficial, as low diversity was associated with more rapid lung function decline over the following 5-year period (29). Perhaps this increased diversity helps to mitigate airway damage directly or by effectively competing against traditional CF pathogens, such as *P. aeruginosa* and *Burkholderia*. Zemanick et al. made similar observations studying a cohort of 136 pediatric and 10 adult patients with CF across several centers (14), with *Streptococcus* spp. more frequently being the dominant microbe among younger subjects (<6 years old) and with an inverse correlation between airway inflammation and the levels of *Streptococcus* spp. being seen. Importantly, these investigators found that the lower levels of inflammatory markers associated with the presence of *Streptococcus* spp. remained even after controlling for the relative abundance of *Pseudomonas* via a multivariate regression analysis; we take these data to suggest that it is the presence of *Streptococcus*, rather than the absence of *Pseudomonas*, that is associated with lower airway inflammation. Finally, metatranscriptome analysis of a cohort of patients with a second disease associated with chronic bacterial infections in the airway, chronic obstructive pulmonary disease (COPD), showed that while patients with the highest levels of transcriptionally active *Streptococcus* spp. had higher bacterial loads, they were less likely to suffer from an exacerbation (39).

Together, these studies indicate that the streptococci may influence disease progression through a mechanism that is not currently understood but that may function by mitigating airway inflammation and/or damage. While the absence of any bacterial

load in the airway is preferred, in the context of CF, these studies suggest in the aggregate that if microbes are present, the less severe outcome appears to be associated with a high relative abundance of streptococci. It is important to note here that we are not suggesting that *Streptococcus* should be considered a probiotic to treat CF-associated infections but, rather, are noting several clinical associations wherein patients with higher loads of *Streptococcus*, compared to other CF pathogens' appear to have a lower disease burden. Of course, given the correlative nature of these studies, it is equally possible that less damaged lungs better support high relative levels of streptococci.

The data presented here, combined with the findings of the studies described above associating SMG members and *S. pneumoniae* with exacerbation in CF, paint a complicated picture of how streptococci impact the host in the context of polymicrobial CF airway infections. Thus, in future studies examining the role of *Streptococcus* spp. in disease, it is of utmost importance to understand which species of these microbes are present, as well as which strains should be chosen for use in any *in vitro* studies.

STREPTOCOCCI IMPACT *P. AERUGINOSA* BIOLOGY, VIABILITY, AND VIRULENCE FACTOR PRODUCTION

There are several studies that indicate that streptococci and *P. aeruginosa* cocolonize patients with CF (1, 2, 8, 10), and thus, these microbes likely have the opportunity to interact with each other, although additional studies are sorely needed to understand the extent of this interaction *in vivo*. *In vitro* studies also demonstrate that mucoid and nonmucoid *P. aeruginosa* strains can coaggregate with a variety of oral streptococci (40, 41), and our group showed that *P. aeruginosa* and *Streptococcus constellatus* could form mixed microcolonies *in vitro* when these microbes formed biofilms on a CF patient-derived airway cell line (42). These data both indicate the potential for these different microbes to physically interact and provide a mechanism whereby these oral microbes might be carried deeper into the airway via their physical association with *P. aeruginosa*. Recent studies have just begun to explore how such interactions might impact the respective microbes in the context of polymicrobial infections, and we review such studies below.

One study demonstrated that an oropharyngeal *Streptococcus* isolate interacts with *P. aeruginosa* through AI-2 signaling and that this signal induces transcription of the *P. aeruginosa* virulence factors elastase, phenazines, and rhamnolipids, resulting in increased *P. aeruginosa*-mediated virulence in a rat agar bead model of chronic lung infection (43). In contrast, the oropharyngeal *Streptococcus* isolate did not contribute to lung disease in monoculture in the rat lung. These results are further supported by additional studies that demonstrate increased elastase, phenazine, and rhamnolipid production by *P. aeruginosa* strains exposed to streptococci, including *Streptococcus anginosus* (44, 45). Interestingly, two *lasR* mutant variants of *P. aeruginosa* from patients with CF showed more robust pyocyanin and elastase production and associated host damage and inflammation when grown in coculture with several SMG members (44), indicating that coculture with another microbial species can (at least in part) bypass the quorum sensing (QS) defect of the *lasR* mutant strains. Analogous observations have been made for *P. aeruginosa lasR* mutants growing in coculture with the fungus *Candida albicans* (46), indicating that the loss of QS signaling observed for pure strains of *P. aeruginosa* isolated from the CF airway may not accurately reflect the situation in the context of an *in vivo*, polymicrobial infection. Furthermore, a recent study also demonstrated that *S. anginosus* can stimulate *P. aeruginosa* to convert from a mucoid phenotype to a nonmucoid, high-pyocyanin-producing phenotype in an *in vitro*, hypercapnic (10% CO₂) environment; this phenotype is associated with the reduced survival of *Galleria mellonella* wax moths, which are used as an insect model of pathogenesis, during infection (47, 48). Taken together, these data call into question the strengths of the conclusions that can be drawn regarding the virulence potential of strains in the CF airway based solely on *in vitro* clinical culture data assaying single species.

In contrast to the positive interaction described above, some interactions between *P. aeruginosa* and streptococci can be detrimental to *P. aeruginosa*. For example, oral streptococci, such as *S. oralis*, *S. sanguinis*, and *S. gordonii*, have been shown to produce H_2O_2 , which can inhibit *P. aeruginosa* when the streptococci are established as primary colonizers (49, 50). Interestingly, these interactions are influenced by environmental conditions, such as (i) aerobic conditions, when *S. oralis* can cocolonize with *P. aeruginosa* (49); (ii) conditions in which streptococci can use H_2O_2 production to outcompete *P. aeruginosa* in 10% CO_2 (49); or (iii) environments wherein *S. parasanguinis*, *S. gordonii*, and *S. sanguinis* can produce H_2O_2 , which reacts with excess nitrite in the medium to form reactive nitrogenous intermediates (RNI) that inhibit *P. aeruginosa* growth (50, 51). Together, these studies indicate that the local environment might play a large role in the outcome of interactions among these microbial species, which is of great relevance to a CF airway, which includes anoxic and hypoxic environments (52–54), as well as the more typical normoxic environment. It is also important to note that there is clearly variability in the observations made regarding interactions as a function of which isolate of *P. aeruginosa* and/or a *Streptococcus* sp. is used; therefore, investigators should strongly consider using multiple isolates of *P. aeruginosa* (mucoid and nonmucoid) and multiple *Streptococcus* species (and isolates of those species) to better understand the generality of any finding related to polymicrobial interactions.

As mentioned above, Scofield and Wu (50) documented that *S. parasanguinis*, *S. gordonii*, and *S. sanguinis* can inhibit the growth of *P. aeruginosa* when provided with nitrite; it is the interaction of the nitrite plus the *Streptococcus*-derived peroxide that results in the generation of RNI. Given the evidence that *P. aeruginosa* participates in dissimilatory nitrogen metabolism in environments like the CF airway (55–57) and the evidence for nitrate and denitrification in CF patient sputum (58–62), there is a clear potential for the generation of RNI in the context of these polymicrobial infections. Further supporting their model, in a subsequent report (51), Scofield and Wu showed that a *P. aeruginosa* strain defective for its nitrite reductase (*nirS*) showed increased sensitivity to nitrite-mediated killing, likely due to the accumulation of nitrite in the culture (51) and, presumably, increased RNI production. Interestingly, these investigators identified a mutation in the permease component of an ABC transporter (PA3252) that was resistant to this killing by *S. parasanguinis* (50), indicating the possibility that the relationship between these microbes has the potential to evolve in the context of chronic, long-term CF airway infections. These investigators did show that *S. parasanguinis* could protect *Drosophila* from a *P. aeruginosa* infection, indicating an important proof of principle that such interactions may indeed occur *in vivo*.

Finally, it is likely that other metabolic interactions in the CF airway might drive changes in the interaction between *P. aeruginosa* and *Streptococcus*. As an example, Flynn and colleagues (63) showed that *P. aeruginosa* can only inefficiently utilize the mucins found in abundance in the CF airway, but they demonstrated that some anaerobes, including *Streptococcus*, as well as *Prevotella* and *Veillonella*, can degrade mucins and generate small fatty acids that can be utilized by *P. aeruginosa* for enhanced growth. Oral streptococci have previously been shown to effectively degrade salivary mucins (64). In particular, Hunter and colleagues, using growth and mutational studies *in vitro* and measuring the gene expression of *P. aeruginosa* in sputum, noted that propionate is one such carbon/energy source produced by anaerobes and utilized by *P. aeruginosa* (63). Indeed, this report and others have detected propionate in lavage and sputum samples from the CF airway (63, 65, 66). There are likely additional such interactions between these (and other) microbes driven by metabolic cross feeding that will be uncovered going forward.

P. AERUGINOSA IMPACTS STREPTOCOCCAL VIABILITY AND GROWTH

Just as streptococci can impact *P. aeruginosa*, the converse is true as well. A recent report from our group demonstrated that *P. aeruginosa* limits *Streptococcus* growth, likely via iron sequestration, in minimal medium coculture conditions (67). Iron availability in the airway has been demonstrated to range from 0.02 μM in healthy controls

to levels as high as 8 μM in CF patients, and the measured patient-to-patient variability is quite high in CF patient sputum (68–71). Furthermore, the extent of iron bioavailable to streptococci is unclear and difficult to determine. Given the potentially high concentration of iron in the CF airway, it is not clear if *P. aeruginosa* and streptococci are competing for iron in the CF lung and, if so, when or where.

P. aeruginosa has also been demonstrated to suppress *S. constellatus* biofilm formation through the secretion of monorhamnolipids and β -hydroxyalkanoyl- β -hydroxyalkanoic acids (HAAs) (42). This suppression of biofilm formation can be alleviated by treatment of CF patients with the maintenance antibiotic tobramycin. These data indicate that the CF airway environment and therapeutics used in the context of CF can also modulate microbe-microbe interactions directly, as described above, and perhaps indirectly via altering the airway environment via modulating iron levels, for example.

In contrast to the negative interactions described above, recent studies have found that *P. aeruginosa* can also enhance *Streptococcus* growth through one or more currently undescribed pathways (42, 44, 47, 48, 67). For example, our group demonstrated that *P. aeruginosa* clinical and laboratory strains can enhance the growth of multiple oral *Streptococcus* spp., including members of the SMG, through a currently unknown mechanism (67). These data indicate that the presence of *P. aeruginosa* can promote the growth and/or persistence of streptococci in the context of the CF airway.

Interestingly, a recent study using a metagenome-based approach argued that *Streptococcus* spp. replicated their genomes (a surrogate for growth) 40- to 60-fold faster than *P. aeruginosa* (72). This finding would be consistent with the published observations that coculture of *Streptococcus* with *P. aeruginosa* enhances the growth of the *Streptococcus* with no obvious benefit to *P. aeruginosa* growth (42, 67).

Furthermore, Scofield and colleagues investigated the increased biofilm formation of *S. parasanguinis* cocultured with a mucoid *P. aeruginosa* strain and found that *S. parasanguinis* is able to bind to *P. aeruginosa*-produced alginate using the streptococcal surface adhesin BapA1 *in vitro* or BapA1 and Fap1 in a *Drosophila melanogaster* coinfection model (73).

Taken together, these studies indicate that, at least in some contexts, *P. aeruginosa* can enhance *Streptococcus* species growth or biofilm formation; however, the mechanisms underlying such interactions are still poorly understood. Thus, a significant effort directed toward understanding the mechanisms underlying *P. aeruginosa*-*Streptococcus* interactions will likely be fruitful going forward.

INTERACTIONS BETWEEN STREPTOCOCCI AND OTHER CF-ASSOCIATED PATHOGENS

Very few studies have investigated the interactions between streptococci and other pathogens in the context of CF. For example, anaerobic Gram-negative bacteria that produce β -lactamases, in particular, *Bacteroides* spp., have been shown to protect GAS from penicillin in a mouse model of infection (74), and thus, it is possible that other β -lactamase-producing microbes could exert a similar protective effect for other streptococci (75, 76).

Additionally, there is a dearth of investigations into multispecies interactions (i.e., the interactions of 3 or more microbes) in the context of CF, but two interesting studies have investigated how polymicrobial interactions among *S. anginosus*, *S. aureus*, and *P. aeruginosa* affect antimicrobial susceptibility in a multispecies biofilm (77, 78). The first study indicates that *S. anginosus* biofilm cells can be protected from cell wall-active antibiotics when grown in a multispecies biofilm with *P. aeruginosa* and *S. aureus* or when *S. anginosus* monoculture biofilms are treated with *S. aureus* supernatant (77), indicating that *S. aureus* is able to protect *S. anginosus* from these antibiotics. Additionally, these investigators demonstrated that *S. aureus* is sensitized to both cell wall-active antibiotics and others antibiotics, like tobramycin, in a multispecies biofilm with *P. aeruginosa* and *S. anginosus* (77). *P. aeruginosa* antibiotic sensitivity is relatively unaffected during polymicrobial culture (77), a finding consistent with the findings of

our studies (79). In a subsequent study, the same team explored the mechanism whereby coculture in a community could render *S. anginosus* biofilm cells protected from the cell wall-active antibiotic vancomycin (78). Tavernier et al. reported (78) that in the presence of *P. aeruginosa*, *S. anginosus* produces a thicker cell wall, which has previously been described to be a mechanism by which *S. aureus* is protected from treatment with vancomycin (80–82). Consistent with this observation, coculture of *S. anginosus* with *P. aeruginosa* resulted in the upregulation of 36 genes in the cell wall synthesis and recycling functional category, the largest such category of genes up-regulated in *S. anginosus* during growth in coculture, compared to their regulation in a monoculture of *S. anginosus*.

Together, these studies indicate that the interactions among streptococci and other CF pathogens can be complex, and the possible outcomes of these interactions in the CF lung microenvironment are not always obvious. However, given that ~30% of patients with CF have both *S. aureus* and *P. aeruginosa* in their airway (83, 84) and SMG members were found to cocolonize patients infected with *P. aeruginosa* and *S. aureus* (2, 7, 85), such polymicrobial interactions are likely not uncommon. Additionally, interactions with streptococci are not limited to the lower airways, and a multitude of studies have investigated polymicrobial interactions with streptococci in the oral microbiome (reviewed in reference 86). These oral microbiome studies may provide some insights into how streptococci could interact with microbes in the CF airway. However, further research is required to fully understand how streptococci might interact with other CF lung pathogens and how these interactions may modulate microbial growth or biofilm formation and/or impact the host airway.

CONCLUSIONS

The role that streptococci play in the CF lung needs to be investigated further, because the current evidence indicates that the predominance of the *Streptococcus milleri* group correlates with exacerbations, while the increased relative abundance of other streptococci may correlate with a lower disease burden in CF patients. It is currently unclear how these complex relationships between streptococci and outcomes in CF patients are mediated.

For the detrimental interactions, it is unclear whether these effects are due directly to the interactions between *Streptococcus* spp. and the host or whether streptococci are able to negatively influence patient health indirectly through interactions with cocolonizing microbes within the lung (Fig. 1). The data thus far indicate that oral streptococci can potentiate virulence factor production of *P. aeruginosa* *in vitro* and *in vivo* (43–45, 47, 48).

In contrast, as described above, younger and healthier patients earlier in the disease stage are found to have a greater diversity in the airway microbiome, including more streptococci, and, thus, a lower abundance of typical CF pathogens. It is possible that the streptococci found in the airway are able to reduce the relative abundance of *P. aeruginosa* through the production of hydrogen peroxide (49) and reactive nitrogenous species (50, 51), as described in *in vitro* coculture studies. Alternatively, oral streptococci may actively reduce inflammation, a conclusion consistent with a recent report (87). Finally, streptococci may exert their impact via a niche exclusion mechanism, that is, by simply preventing high-level colonization of the airway by *P. aeruginosa* or other CF pathogens. Additional studies are required to determine the nature of the influence that the streptococci have on the CF airway.

Finally, it is currently unknown what kinds of interactions streptococci may have with CF lung pathogens other than *P. aeruginosa*, such as *S. aureus*, *Stenotrophomonas*, *Burkholderia*, or others. All of these interactions can potentially impact host health, as well as the responsiveness of these microbes to therapeutics. It is clear that for polymicrobial interactions, such as those in CF patients, that we must move beyond the one-bug mind-set. The observation that streptococci can have differential impacts on patient outcomes adds an additional layer of complexity to any analysis. We would argue, however, that the large available data sets on the airway microbiota in CF

patients during clinical stability and disease exacerbation, plus the rich clinical data available, provide a unique opportunity to study polymicrobial interactions and their impact on the host using streptococci as model organisms.

Given the well-documented nature of polymicrobial infections in the context of CF (1–3), we suggest that interactions of streptococci with other CF-related pathogens is an emerging model system that may be used to provide an understanding of the molecular mechanisms of microbe-microbe interactions; such studies can exploit the abundant extant data regarding the environmental, nutritional, and microbial context of the CF lung. While other interactions have been studied in depth, particularly between *P. aeruginosa* and *S. aureus* and their negative impacts on CF patients (84), we see that one advantage of using streptococci as a model system is, as described above, the participation of these microbes in processes with varied impacts on the host. We also argue that the most productive approach to dissecting such interactions is to leverage multiple techniques, including microbiome studies, *in vitro* models, clinical studies, the latest omics (metagenomics, metabolomics, proteomics) technologies, and modeling, and such data have been generated for sputum, lavage, and brush samples from the CF airway. Finally, the ability to generate complementary data from laboratory experimental models and the analysis of large clinical data sets make such an approach particularly powerful.

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