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Early Antibiotics in Cystic Fibrosis: Lessons from the Cystic Fibrosis Pig Model

Cystic fibrosis (CF) is caused by mutations in CFTR (cystic fibrosis transmembrane conductance regulator), which results in defects in ion transport. The leading causes of morbidity and mortality are respiratory symptoms and progressive pulmonary failure. Three hallmarks of this pathogenesis identified to date are abnormal mucus accumulation, mucus tethering, chronic sinopulmonary inflammation, and recurrent infections (1). However, there has been continued debate about what comes first: infection or inflammation, akin to the chicken or the egg argument.

Evidence of intrinsic airway inflammation has been described in fetal lungs homozygous for the $\Delta F508\text{del}$ -CFTR mutation. This study found that even in fetal lung tissue, which was presumably sterile, there was overexpression of proinflammatory proteins and evidence of nuclear factor- κB activation in the airway (2). Meanwhile, studies from AREST-CF (the Australian Respiratory Early Surveillance Team for Cystic Fibrosis) showed that bacterial infections can exacerbate airway inflammation and worsen other clinical outcomes in early CF lung disease as well (3, 4). Newborn screening allows us to diagnose CF and apply early interventions before clinical presentation. Early childhood may represent a critical time point to delay or prevent the onset of lung damage and may impact the future clinical trajectory (5). In this issue of the *Journal*, Bouzek and colleagues (pp. 692–702) used the CF pig model to study bacterial-dependent and -independent inflammatory responses and mucus accumulation in newborn pigs (6).

The authors chose the CF pig model because this model develops spontaneous lung disease. Commonly used rodent models are not ideal in the CF lung field as they fail to develop spontaneous lung disease as observed in humans (7). Investigators have developed newer animal models for CF, including pigs (8), ferrets (9), and rabbits (10), that to some extent overcome this limitation. The newborn pigs in this paper have similar anatomical, physiological, and biochemical features to those of early events in humans with CF compared with other models, such as 1) pigs contain submucosal

glands throughout the cartilaginous airways, whereas those in rodents are limited in trachea; 2) the major type of secretory cell is the goblet cell instead of the club cell in mice; and 3) newborn pigs with the CFTR mutation have acidic airway surface liquid pH (11) and mucous tethering (12), leading to lower bacterial killing efficiency and abnormal mucociliary clearance. Within months, they develop spontaneous sinopulmonary diseases with hallmarks of CF such as infection, inflammation, mucus accumulation, airway remodeling, and lobar pathological heterogeneity (13).

To determine the effects on airway bacterial burden as well as airway inflammation, the authors applied early-onset continuous broad-spectrum antibiotics to newborn CF pigs. Before the initiation of antibiotics, CF pigs at 3 weeks of age had a greater absolute number of bacteria as well as a greater number of bacterial species in lung tissue than non-CF pigs. Continuous antibiotic treatment (a combination of ceftiofur hydrochloride intramuscularly from birth as well as oral cephalixin, ciprofloxacin, and trimethoprim-sulfamethoxazole till study completion) reduced the bacterial burden as well as the bacterial species abundance in lung tissue. Furthermore, antibiotic treatment reduced certain aspects of CF lung pathology, including less mucus accumulation, measured by periodic acid–Schiff staining, and less lung parenchymal heterogeneity, measured by computed tomography (CT) lung scanning, compared with control CF pigs. However, some lung abnormalities persisted. Antibiotic-treated CF pigs still showed similar inflammatory histopathologic scores in hematoxylin and eosin staining and air trapping abnormalities on CT imaging.

Notably, this extensive antibiotic regimen did not affect sinusitis, with no reduction in bacterial burdens in this anatomic niche. However, the authors did find that antibiotics altered the dominant bacterial species from *Streptococcus* spp. to *Enterococcus* spp. and *Pseudomonas* spp., which may suggest an active selection mechanism. This shift in bacterial species induced by antibiotics was also observed in the lungs and had a strong correlation with lung heterogeneity on CT imaging. This raises concerns for the use of routine clinical antibiotics as the main option in treating or preventing frequent infections in CF (14), given the potential risks of antibiotic resistance, disruption of gastrointestinal tract microbiome, and bacterial selection. In turn, this shift in bacterial populations may further contribute to the persistent sinopulmonary disease. Stutman and colleagues demonstrated higher colonization of *P. aeruginosa* and no improvement on the major health outcomes after continuous antistaphylococcal antibiotics prophylaxis (15).

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Going back to the debate about infection and inflammation, what have we learned? Loss of CFTR leads to multiple host defense defects. The CFTR defect alone can alter the gut microbiota and increase T-helper cell type 17 signaling in the mesenteric lymph nodes and spleens in a germ-free CF mouse model, which has a comparable intestinal histopathologic score to CF mice raised in a laboratory facility with lab-based gut flora (16). Infection adds more variables or stimuli to these complex pathologies. Airway mucus plugging frequently occurs in infants with CF and newborn pigs before detectable bacterial infection (17). These mucous abnormalities appear to not be reversed by the antibiotic treatment, which is one possible explanation for airway inflammation independent of infection (18). In support of this, CF ferrets still develop structural bronchiectasis and neutrophilic airway inflammation without detectable bacterial infection (19). Similarly in this paper, continuous antibiotic treatment reduced the bacterial burden but did not eliminate airway inflammation. A limitation of the present study is the reliance on culture, as culture-independent techniques may be more sensitive to identify microbial components such as cell wall components, microbial DNA, phage, or metabolites. These components may be important drivers of inflammation as even nonviable bacteria can stimulate many inflammatory signaling pathways. Certainly, we cannot understate the contributions of bacterial infection to respiratory disease in CF. They are tightly associated with lung inflammation, exacerbation, and lung function loss over time.

In an era of CFTR modulators, the quality and longevity of life for patients with CF have been improved greatly. As the clinical studies continue to be performed in younger individuals, it will be critical to understand how these drugs impact mucous clearance, airways inflammation, and infection. Although this paper focused on the contributions of bacterial infection, we know there is a complex interplay between viral infection and bacterial infection and specifically bacterial biofilm formation (20) and that this will be an important area to model in preclinical models. This paper highlights the potential need to target inflammation independent of infection to have the greatest impact in preserving lung function in patients with CF. ■

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