Membrane Lipids and CFTR: The Yin/Yang of Efficient Ceramide Metabolism

Understanding the pathophysiology associated with deficient CFTR (cystic fibrosis transmembrane conductance regulator) function in cystic fibrosis (CF) requires a systematic approach with the integration of physiology, epigenomes, metabolism, and structural integrity cells into complex tissues and organ function. Cell membranes contain sphingomyelin, cholesterol, proteins, and water, which provide the initial encounter with the constantly changing surrounding milieu (1). Sphingolipid metabolism, at the center of this exchange, is anchored on ceramide as an intermediate in the steps toward sphingosine (2). Ceramide functions in the structural integrity of cell membranes, which, together with cholesterol and other lipids, participate in insulating and transducing cellular responses associated with differentiation, proliferation, and programmed cell death (3).

Pursuing the role of ceramide in CF requires attention to the details in resources, sample processing, experimental design, and highly sensitive technology (4). Analyzing ceramide is further complicated depending on whether synthesis is *de novo* and/or through sphingomyelin hydrolysis (2). Because the catabolic process of sphingomyelin to sphingosine is dynamic and reversible, stringency in experimental conditions is an important contributor to experimental outcomes. The distribution of ceramide versus sphingomyelin and sphingosine contributes to the delicate balance of antiinflammatory function versus antimicrobial efficiency in the host response to infection (2, 5, 6). Upon challenge by pathogens, sphingomyelin is processed into ceramide, which is further processed to sphingosine in a series of enzymatic steps, including sphingomyelinases and acid ceramidase. The change in sphingomyelin/ceramide/sphingosine distribution affects the biophysical properties of the membrane, impacting cell signaling, structure, and the homeostatic balance. Post–pathogen exposure, sphingomyelin hydrolysis to ceramide in T cells regulates T-cell cytoskeletal reorganization for motility, antigen presentation, and cell–cell interactions (6–8). Elevated ceramide in CF epithelial cells and alveolar macrophages (in mice and humans) has been associated with aberrant cellular apoptosis, impaired mucocillary clearance, and susceptibility to infection and chronic inflammation (5, 9).

In this issue of the *Journal*, Gardner and colleagues (pp. 1133–1145) used mass spectrometry technology and a series of *in vitro, in situ* CF tracheal and *in vivo* murine models to follow the sphingomyelin hydrolysis in the CFTR-deficient epithelial cells compared with CFTR sufficient control cells (10). These studies quantified sphingomyelin, ceramide, and sphingosine profiles with changes in response in the presence and absence of gram-negative (*Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) pathogens. The associated shift in the sphingomyelin, ceramide, and sphingosine with these different pathogens are important because they have unique exchanges with the cell membrane. The studies demonstrated that sphingomyelin, in the presence and absence of pathogen exposure, was abnormally processed in CFTR-deficient scenarios, with the efficient production of sphingomyelinase and processing to ceramide but the accumulation of ceramide because of deficient concentrations of acid ceramidase. The inefficient processing of ceramide contributed to deficient levels of sphingosine, correlating with the inefficient management of infection and inflammation. The disproportionate relationship between the sphingomyelin, ceramide, and sphingosine correlated with enhanced susceptibility to pulmonary infections, and inflammation was further associated with increased TNF receptor 1 expression, NFκB activation, and proinflammatory cytokines *in vivo* associated with CF *P. aeruginosa* infection.

Farber disease is a lysosomal storage disorder associated with deficient acid ceramidase activity (2, 11), which is treated with recombinant human acid ceramidase (11, 12). Gardner and his colleagues used recombinant human acid ceramidase to treat the deficient hydrolysis of ceramide in their models and demonstrated improved management of both infection and inflammation *in vitro* and *in vivo*. The observed double benefit of pathogen and inflammation management is important in the context of distinguishing deficient epithelial response to pathogens and altered downstream capacity to manage infection and inflammation. Acid ceramidase treatment decreased ceramide and enhanced sphingosine production with a concomitant decreased number of neutrophils, macrophages, and proinflammatory cytokines and did not enhance bacterial colonization, an important consideration in scenarios of chronic infection.

It would have been valuable to explore the role of sphingomyelin metabolism in other CFTR mutation combinations because variations in CFTR structure/function may have relative differences in sphingomyelin processing and the efficiency of ceramide hydrolysis. Cholesterol transporters (13) or other related genetic modifiers may also impact the sphingomyelin systematic response to pathogen exposure (14). In lysosomal storage diseases such Niemann-Pick disease, the accumulation of sphingomyelin and deficiency of ceramide are both related to deficient acid sphingomyelinase or a defective cholesterol transporter (14). Modifier studies have certainly implicated complexities associated with the downstream effects of the CFTR deficiency. Understanding the intricacies in sphingomyelin hydrolysis and CFTR mutations may shed light on the direct role of CFTR on metabolism and the production of sphingosine from ceramide. It is important to understand the impact of the ceramide accumulation and/or sphingosine deficiency on normal cellular functions because specific relationships between sphingomyelin, ceramide, and sphingosine ratios have not been interrogated in great detail with respect to cell immune function, which may be more relevant than ever in the era of modulator therapy and improved survivability

Originally Published in Press as DOI: 10.1164/rccm.202006-2362ED on July 20, 2020

of patients with CF. The fact that the inhibition or the genetic ablation of genes controlling sphingolipid synthesis have been associated with hepatic steatosis suggests that the long-term effects of sphingolipid metabolic deficiencies may become associated with excessive hepatic triglyceride accumulation (16).

How are CFTR mutations linked to deficient sphingomyelin hydrolysis, accumulated ceramide, deficient acid ceramidase, and decreased sphingosine? Answers to this question may provide important information about the essential role of sphingomyelin metabolism in cell membrane function in CF. Furthermore, the impact of lipid accumulation may pose an important insight into CFTR pathophysiology beyond that which is addressed by current modulator therapy, contributing to therapeutic outcomes. Gardner uses innovative technology to explore the role of sphingomyelin, ceramide accumulation, and deficient sphingosine production in cellular function and pathophysiology in CF. The use of recombinant acid ceramidase as a potential antiinflammatory therapeutic makes sense from a basic biological standpoint, associating the intricacies of cellular design and the impact of deficient CFTR on cell function. The answer lies in the details of understanding CFTR mechanistically beyond ion transport in the hunt for management of all of the disease-associated pathologies for individuals who suffer from CF. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Revisiting the Role for HIF Stabilizers in Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD), a form of chronic lung disease, is a severe complication for newborns born prematurely, particularly for those born at extremely low gestational ages. The physiopathology of BPD remains incompletely understood but can be summarized by the consequence of various perinatal injuries, such as infection, mechanical ventilation, and oxygen therapy, on a predisposing field: the immature lung (1). At the histological level, BPD is primarily characterized by airspace enlargement due to defective alveolar formation, a process tightly regulated by a complex and mutually dependent relationship among pulmonary epithelial, interstitial, and endothelial cells (2). Although the past decade has allowed for improved survival of infants with extremely low gestational age, owing to antenatal glucocorticoid treatment, surfactant therapy, and less injurious neonatal resuscitation strategies, the incidence of BPD has not been reduced, and BPD, often complicated by pulmonary hypertension (PH), continues to be a major challenge for neonatologists and pediatricians. The identification of new actionable targets to prevent and treat BPD is thus a major research priority, and this is especially true as a growing body of research evidence is now available describing survivors of BPD as a population exhibiting poor neurodevelopmental outcomes

Supported by NIH grant R01-HL-134802 (E.D.A.).

Originally Published in Press as DOI: 10.1164/rccm.202006-2570ED on July 30, 2020