Bioactive Sphingolipids in the Pathogenesis of Chronic Obstructive Pulmonary Disease

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Abstract

A better understanding of the pathogenesis of distinct chronic obstructive pulmonary disease (COPD) phenotypes will improve diagnostic and therapeutic options for this common disease. We present evidence that sphingolipids such as ceramides are involved in the emphysema pathogenesis. Whereas distinct ceramide species cause cell death by apoptosis and necroptosis, cell adaptation leads to accumulation of other sphingolipid

metabolites that extend cell survival by triggering autophagy. Cigarette smoke–released sphingolipids have been involved in both the initiation and persistence of lung injury via intracellular signaling and paracrine effects mediated via exosomes and plasma membrane–bound microparticles. Strategies to control sphingolipid metabolite production may promote cellular repair and maintenance to treat COPD.

Keywords: ceramide; sphingosine-1 phosphate; emphysema

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Chronic obstructive pulmonary disease (COPD) is a heterogeneous condition that comprises multiple phenotypes and endotypes and that is primarily induced in susceptible individuals by chronic exposure to cigarette smoke (CS). A better understanding of the pathogenesis of distinct COPD phenotypes will lead to an improved diagnostic and therapeutic portfolio for this prevalent disease. The mounting evidence that sphingolipids are involved in COPD pathogenesis prompts further mechanistic studies to elucidate the contribution of this class of lipids to the risk of injurious effects of CS, as and to the initiation and persistence of this disease. In this report, we highlight current understanding of sphingolipid involvement in COPD, with a focus on studies contributed by our laboratory. Our

first appreciation of the importance of sphingolipids in COPD pathogenesis was in the context of the loss of alveolar tissue in emphysema due to loss of vascular endothelial growth factor (VEGF) signaling (1). Whereas emphysema is characterized by a net loss of alveolar capillaries in the setting of a pauci-VEGF state (2, 3), the other major COPD phenotype, chronic bronchitis, is characterized by airway hypervascularity with leaky angiogenesis of bronchial vessels and excessive VEGF (4). These two distinct vascular pathologies are likely to have different pathogenesises influenced by unique local factors in the lung parenchyma and the large airways. To better understand which signaling or cellular response pathways are associated with distinct known COPD phenotypes, we have applied unbiased

metabolomic and genomic approaches to biological samples from subjects enrolled in COPDGene (COPD Genetic Epidemiology). This study included well-phenotyped individuals with COPD as well as smokers and ex-smokers without a diagnosis of COPD. One of the top pathways significantly associated with COPD at both metabolite and gene levels was the sphingolipid metabolism pathway (5).

Sphingolipid Metabolism

The sphingolipid metabolism is centrally anchored on ceramide. “Ceramide” is a generic term for multiple ceramide species with distinct fatty acids linked to a sphingoid backbone, of which palmitoyl and

lignoceroyl ceramides are the most abundant in the lung (6). Ceramide is centrally positioned in the sphingolipid metabolism because it can be generated either from *de novo* synthesis or from sphingomyelin hydrolysis, and then used as building block for the synthesis of complex sphingolipids. The dynamic course of sphingolipid metabolism is governed by multiple enzymes anchored in particular organelles. From there, metabolites can either cross membranes or be transported by specialized proteins to other cellular compartments. Besides structural roles, several sphingolipid metabolites have been named “bioactive molecules” owing to signaling activities, for example as second messengers. A simplified schematic of the sphingolipid metabolism is depicted in Figure 1A.

Role of Sphingolipids in Homeostasis

Our previous work has demonstrated that the balance between intracellular levels of ceramide and its downstream metabolite sphingosine-1-phosphate (S1P) is crucial for lung cell fate (Figure 1B). The proapoptotic effect of excessive ceramide can be prevented or mitigated by approaches that increase S1P in murine lungs (7). This concept is not unique to the lung or to COPD, because this relationship between ceramide and S1P is required for the maintenance and health of multiple tissues, as evidenced by publications reviewed elsewhere (8). The production of ceramide itself is of utmost importance to the baseline

cellular function. Even brief inhibition of the lysosomal acid sphingomyelinase activity promptly destabilizes the lysosomal nutrient-sensing complex, inhibits mammalian target of rapamycin signaling, and triggers autophagy with preserved lysosomal degradation (9). Furthermore, long-standing inhibition of acid sphingomyelinase causes the lipid storage Niemann-Pick disease, in which pulmonary phenotypes are increasingly being recognized (10, 11). These findings suggest that an optimal concentration of ceramide and its bioactive sphingolipid metabolites is paramount for proper tissue function. Understanding the precise biochemical regulation of ceramide and S1P content in response to CS exposure that culminates in emphysema may reduce excessive structural lung cell apoptosis and maintain lung tissues, mitigating the severity of this disease.

To dissect the sphingolipid metabolic derangements in emphysema lungs, we have evaluated frozen lung tissues collected by the Lung Tissue Research Consortium from individuals with COPD. Studies of human lung enzyme activity indicated an inverse correlation between the activity of the S1P-producing sphingosine kinase 1 with the severity of emphysema as determined by quantitative assessment of thoracic computed tomographic scans ($r = -0.42$, $P < 0.0001$). These results suggested that lung cells might not appropriately compensate for increased proapoptotic ceramides with an adequate increased S1P synthesis. Furthermore, a meta-analysis of sphingolipids associated with emphysema using distinct mass spectrometric approaches showed a significant inverse correlation between plasma sphingomyelin and emphysema, as well as between the globoganglioside (GM3) glycosphingolipids and emphysema (5). Data obtained from these translational studies led us to pursue mechanistic studies in reductionist models of emphysema using relevant primary human cell cultures exposed to CS extract or murine models of CS exposure.

Sphingolipids in Early Responses to CS

Investigations of the role of sphingolipids in early cell injury responses to CS in primary human lung microvascular endothelial cells

Figure 1. (A) Schematic of sphingolipid metabolism. Highlighted are the enzymes responsible for the steady- or conditional-state levels of S1P: SphK1, or SphK2, SPL, and SPP. SphK1 and SPL also catabolize DH-sphingosine produced in the *de novo* pathway of sphingolipid synthesis (initiated by SPT). Also shown are upstream metabolites in the sphingolipid pathway that can accumulate intracellularly if SphK1 is inhibited. (B) Schematic illustrating how ceramide/S1P rheostat may be disrupted by CS, which inhibits intracellular S1P production and extracellular signaling via S1PR1, thereby weakening prosurvival effects that oppose ceramide-induced cell death by necroptosis. Excessive lung microvascular cell death, in turn, contributes to emphysema development. ALD = aldehydes; ASAH = acid ceramidases; CERS = ceramide synthases; CoA = coenzyme A; CS = cigarette smoke; DEGS = dihydroceramide desaturases; DH = dihydro-; S1P = sphingosine-1-phosphate; S1PR1 = sphingosine-1-phosphate receptor 1; SM = sphingomyelin; SMases = sphingomyelinases; SphK = sphingosine kinase; SPL = sphingosine-1-phosphate lyase; SPP = sphingosine-1-phosphate phosphatase; SPT = serine palmitoyl transferase.

and mouse and rat lungs revealed their significant involvement in the loss of endothelial cell barrier function (12–14). Both oxidative stress and nicotine led to upregulation of ceramide and to cytoskeletal changes that culminated in endothelial barrier dysfunction as well as a loss of proliferative properties of primary lung microvascular endothelial cells (13, 14). In contrast, gain of function of S1P, both directly and through activation of S1P1, a S1P receptor on endothelial cells, led to alleviation of endothelial barrier dysfunction and to restoration of cell-proliferative capacities, which may be important in the repair phase of CS injury (14).

Visualization with two-photon excitation microscopy of leukocyte adhesion and entrapment in lung microvascular spaces following exposure to CS supported the *in vivo* relevance of sphingolipid-related barrier-disruptive effects of CS (12). Over time, these repetitive insults may contribute to peripheral lung injury and improper repair causing lung tissue remodeling characteristic of COPD.

In addition to oxidative stress, improper transportation of S1P intracellularly via the adenosine triphosphate-binding cassette transporter cystic fibrosis transmembrane conductance regulator (CFTR) may also disrupt the endothelial barrier after CS exposure. CFTR-deficient lungs had increased leukocyte adhesion in the lung microvasculature after CS (15). Because CS inhibits CFTR expression and function (16), which in turn disrupts the ceramide–S1P balance in both bronchial and pulmonary endothelial cells (15, 17), the CS-mediated inhibition of CFTR may be instrumental in large airway inflammation, aberrant angiogenesis, and aseptic inflammation in the COPD lungs.

Sphingolipids in Amplification of Responses to CS

A large body of work from our and other laboratories supports an important role of sphingolipids in the amplification of CS-induced injury, such as oxidative stress, apoptosis, and inflammation. Reactive oxygen species generated by CS and VEGF receptor inhibition engage in a feedforward loop between ceramide synthesis, inhibition of antioxidant enzymes such as superoxide

dismutase, and accumulation of superoxide radicals, which further activates ceramide production via acid sphingomyelinase (18). However, cells deploy adaptive mechanisms to minimize the deadly accumulation of ceramide metabolites. For example, cells respond to acute and subacute hypoxia by minimizing the *de novo* ceramide synthesis at the expense of accumulating upstream metabolites such as dihydroceramide (DHC). In turn, DHC induces autophagy and decreases structural lung cell proliferation (19, 20). While engaging adaptive survival mechanisms, DHC may impair the overall ability of lung tissue regeneration and maintenance. We identified such an adaptive response in human lung microvascular endothelial cells isolated from smokers that exhibited an apoptosis-resistant phenotype with more robust autophagy induction in response to stress when compared with cells from nonsmokers (21). Despite their enhanced survival ability, these cells may be less angiogenic than primary cells isolated from smokers (unpublished observation), suggesting that increased survival is attained at the expense of decreased cell repair mechanisms. Whereas molecules increased by CS exposure, such as DHC, initiate autophagy, for as yet unclear reasons, the completion of autophagy via lysosomal fusion and degradation of autophagosome cargo is impaired by CS. This defect renders the adaptive mechanism of autophagy ineffective and contributes to cell death. For example, proper completion of autophagy is required for coping with CS-induced mitochondrial damage via mitophagy; otherwise, cell necroptosis ensues (Figure 1B), a process that, in bronchial epithelial cells, may involve the accumulation of palmitoyl ceramide (20). Indeed, enhancing autophagy with preserved lysosomal degradation via inhibition of acid sphingomyelinase alleviated necroptosis in this model of CS-induced injury (20).

Other cells, such as alveolar macrophages, are more resistant at baseline to the proapoptotic effects of ceramide accumulation. Instead, excessive ceramide decreases their ability to phagocytose apoptotic cells (efferocytosis) by impairing cytoskeletal functions (22). In turn, S1P signaling is implicated in alveolar macrophage phagocytic function (23). This is relevant, since alveolar macrophages from subjects with

COPD are deficient in their ability to phagocytose apoptotic airway epithelial cells (24). Furthermore, studies that built on the established role of S1P gradient in guiding lymphocyte trafficking indicated that S1P receptors are linked to lung lymphocyte infiltration and CS-induced emphysema-like manifestations in mice (25).

Additional amplifying effects of sphingolipids are the result of paracrine effects of extracellular presence of bioactive lipids. Inhibition of lung apoptosis by systemic administration of anticeramide antibodies in the VEGF receptor inhibition model of emphysema provided the first indication of a paracrine or autocrine activity of excessive ceramide (1). The increased ceramide in the extracellular milieu, including the systemic circulation, may be the result of microparticles and exosomes released from CS-injured cells. This release required activation of acid sphingomyelinase, the activity of which was found to be increased in smokers' plasma (26). Ceramide-rich membrane-bound exosomes and microparticles found in the systemic circulation can easily be taken up by and impact the function of uninjured cells. For example, we have shown that uptake of CS-released exosomes inhibits splenic macrophage efferocytosis (26), which, in turn, can augment the systemic inflammatory response to CS. Other derangements in the sphingolipid metabolism stimulate the arachidonic acid metabolic pathway (27) and provide additional mechanisms by which to amplify inflammatory responses.

Conclusions

There is an increasing body of evidence that distinct bioactive sphingolipids play an important role in the dynamic processes of injury, adaptation to injury, repair, and resilience of the lung after CS exposure. Aberrations in sphingolipid metabolism contribute to initiation and amplification of pathogenic processes culminating in emphysema. Sphingolipid-mediated paracrine and endocrine effects may even be relevant to the development of COPD comorbidities. This knowledge can be used in designing phenotype-specific approaches of repairing COPD lungs. Such approaches should aim to

interrupt self-perpetuating lung injury and to reengage lung maintenance programs such as repair, regeneration, and survival. In that regard, targeting the ceramide/S1P rheostat may be a novel strategy for

COPD therapy. By understanding the enzymes that control the ceramide-S1P balance and the relative importance of S1P receptors in COPD pathogenesis, we can develop therapeutic tools to prevent

and/or decrease the severity of lung injury caused by CS. ■

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

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