Crystal Deposits in Macrophages and Distal Lung Remodeling: A Tale of Aging in SFTPC-Deficient Mice

One of the most widespread presentations of interstitial lung disease (ILD) is idiopathic pulmonary fibrosis (IPF), a chronic, progressive, and fatal disease. The prevalence of IPF in the United States has increased twofold in the last 10 years, affecting approximately 180,000 Americans (1). Central to the pathogenesis of IPF is injury to alveolar type II cells, concomitant with mesenchymal cell activation and immune cell dysregulation resulting in enhanced extracellular matrix deposition and lung remodeling (2). Important risk factors associated with IPF include a history of smoking and advanced age (1). Mutations of surfactant genes, SFTPC and SFTPA, have been linked with adult and childhood ILD, where common mechanisms include endoplasmic reticulum (ER) stress, protein aggregation, and apoptosis as a result of production of mutant forms of SFTPC (3). This may be best exemplified by elegant studies where mice carrying a mutant form of SFTPC developed spontaneous lung fibrosis (4). Equally as important as mutant forms of SFTPC is the loss of SFTPC that has been associated with familial ILD (5). In this regard, loss of SFTPC (and other surfactant proteins) after bleomycin-induced lung injury (6) has been reported. In addition, exacerbated inflammatory (7) and fibrotic responses (8) in SFTPC$^{-/-}$ mice have also been reported. However, the chronic effects of SFTPC deficiency on lung injury are not fully understood.

In this issue of the *Journal*, Ruwisch and colleagues (pp. 466–478) link SFTPC depletion with aging and demonstrate that despite early developmental defects, including slower body weight gain, reduced lung volume, inflammatory cell infiltration, and deficits in lung function, mice are able to compensate by 30 to 40 weeks of age (9). Regardless of this recovery, exemplified by improved lung function parameters and reduced inflammatory cell infiltration, histopathological features of lung injury are prevalent, some as early as 10 weeks of age. These alterations in pulmonary architecture include airspace enlargement that appears to precede evidence of fibrotic injury that is detected between 50 and 60 weeks of age. These observations are significant, because they demonstrate that despite early deficits in lung function in SFTPC$^{-/-}$ mice, these animals are able to adapt and present with normal lung function (compared with age-matched SFTPC^{+} mice). Although normal lung function values were present, evidence of fibrotic lung injury became apparent histologically between 50 and 60 weeks of age that correlated with reductions in percent oxygen saturation. Despite these compelling observations, the authors missed an opportunity to longitudinally track markers of senescence, such as β-galactosidase or γ-H2AX, that may help to further understand the link between aging and SFTPC deficiency in mice.

Perhaps the most striking finding was the presence of crystals in alveolar macrophages in SFTPC$^{-/-}$ mice; these novel findings using EM correlated with evidence of crystals in macrophages from IPF lungs. Interestingly, the accumulation of crystals in alveolar macrophages was observed starting at Week 30, once airspace enlargement was present but preceding fibrotic injury. Further experiments revealed that the presence or accumulation of these crystals in alveolar macrophages correlated with altered cholesterol metabolism in macrophages. Herein, Ruwisch and colleagues (9) report a dramatic reduction in the expression of macrophage cholesterol efflux transporters Abca1 and Abcg1 that appear to lead to cholesterol accumulation in alveolar macrophages starting at Week 40, preceding evidence of fibrotic injury in SFTPC-deficient mice. In line with this, lipid-laden macrophages (foam cells) have been demonstrated to promote lung fibrosis (10). In those studies, experimental fibrosis induced by silica, bleomycin, or irradiation resulted in the release of lipid-rich products from alveolar epithelial cells, leading to the accumulation of foam cells in close proximity to the injured epithelium (10). Furthermore, fibrosis was exacerbated in mice lacking the Abcg1 transporter, consistent with Ruwisch and colleagues (9), where SFTPC$^{-/-}$ presented with reduced macrophage Abcg1 expression. Intriguingly, this accumulation of cholesterol in macrophages was observed in both SFTPC$^{-/-}$ and SFTPC^{+} mice where no lung injury was reported. This observation suggests that accumulation of cholesterol alone by macrophages may not be sufficient to promote lung injury but that loss of SFTPC function may be an important trigger that leads to lung injury. Surfactant lipid composition is highly complex, and its different constituents are able to modulate distinct metabolic and inflammatory effects (11). Thus, it is possible that the loss of SFTPC alters the surfactant lipid composition in such a way to promote the development and activation of foam cells that in turn promote fibrotic deposition (10). Although Ruwisch and colleagues (9) demonstrate that cholesterol levels lead to differential update of SFTPC, further experiments are needed to identify how SFTPC deficiency alters the alveolar type II cell–macrophage paracrine lipid axis to promote fibrosis.

The article by Ruwisch and colleagues (9) may have important repercussions to other diseases, such as combined pulmonary fibrosis and emphysema (CPFE), a syndrome identified in 2005 and characterized by coexistence of areas of airspace enlargement, typically in the upper lobes, and areas of fibrotic matrix deposition, predominantly in the lower lobes (12). Although mechanistic studies on CPFE are limited, recent studies have demonstrated increased adenosine signaling and hyaluronan synthesis in macrophages in an experimental model replicating features of

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CPFE and in lung tissues from CPFE lung explants (13). In these studies, inhibition of hyaluronan synthases by hymecromone was able to halt fibrotic lung injury but had no effect on airspace enlargement (13). These observations are significant, because hyaluronan has been reported to form complexes with low-density lipoprotein and induce cell infiltration of foam cells (14), which have been shown to drive lung fibrosis (10). However, the link between SFTPC deficiency, hyaluronan synthesis, and cholesterol accumulation in the pathogenesis of chronic lung diseases such as CPFE and ILD has not yet been fully investigated.

Moreover, the study by Ruwisch and colleagues (9) may also have important ramifications for bronchopulmonary dysplasia (BPD), a disease of preterm infants characterized by stunted development of the alveoli leading to lifelong disease (15). BPD is characterized by inadequate surfactant lipid coating of the alveoli, leading to breathing difficulties that can be treated by supplemental surfactant therapy. However, despite early rescue therapies, many preterm patients develop chronic lung diseases (15). Ruwisch and colleague's research may provide an important link between surfactant deficiency and foam cell activation that may help explain the development of chronic lung injury in patients who survive the early phases of BPD (9). ■

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References

1. Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis. *N Engl J Med* 2018;378:1811–1823.
2. Selman M, Pardo A. Alveolar epithelial cell disintegrity and subsequent activation: a key process in pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:119–121.
3. Mulugeta S, Nureki S, Beers MF. Lost after translation: insights from pulmonary surfactant for understanding the role of alveolar epithelial dysfunction and cellular quality control in fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* 2015;309:L507–L525.
4. Nureki SI, Tomer Y, Venosa A, Katzen J, Russo SJ, Jamil S, *et al*. Expression of mutant Sftpc in murine alveolar epithelia drives spontaneous lung fibrosis. *J Clin Invest* 2018;128:4008–4024.
5. Amin RS, Wert SE, Baughman RP, Tomashefski JF Jr, Nogee LM, Brody AS, *et al*. Surfactant protein deficiency in familial interstitial lung disease. *J Pediatr* 2001;139:85–92.
6. Lutz D, Gazdhar A, Lopez-Rodriguez E, Ruppert C, Mahavadi P, Günther A, *et al*. Alveolar derecruitment and collapse induration as crucial mechanisms in lung injury and fibrosis. *Am J Respir Cell Mol Biol* 2015;52:232–243.
7. Glasser SW, Maxfield MD, Ruetschilling TL, Akinbi HT, Baatz JE, Kitzmiller JA, *et al*. Persistence of LPS-induced lung inflammation in surfactant protein-C-deficient mice. *Am J Respir Cell Mol Biol* 2013; 49:845–854.
8. Lawson WE, Polosukhin VV, Stathopoulos GT, Zoia O, Han W, Lane KB, *et al*. Increased and prolonged pulmonary fibrosis in surfactant protein C-deficient mice following intratracheal bleomycin. *Am J Pathol* 2005; 167:1267–1277.
9. Ruwisch J, Sehlmeyer K, Roldan N, Garcia-Alvarez B, Perez-Gil J, Weaver TE, *et al*. Air space distension precedes spontaneous fibrotic remodeling and impaired cholesterol metabolism in the absence of surfactant protein C. *Am J Respir Cell Mol Biol* 2020;62:466–478.
10. Romero F, Shah D, Duong M, Penn RB, Fessler MB, Madenspacher J, *et al*. A pneumocyte-macrophage paracrine lipid axis drives the lung toward fibrosis. *Am J Respir Cell Mol Biol* 2015;53:74–86.
11. Fessler MB, Summer RS. Surfactant lipids at the host-environment interface: metabolic sensors, suppressors, and effectors of inflammatory lung disease. *Am J Respir Cell Mol Biol* 2016;54: 624–635.
12. Cottin V, Nunes H, Brillet PY, Delaval P, Devouassoux G, Tillie-Leblond I, *et al*.; Groupe d'Etude et de Recherche sur les Maladies Orphelines Pulmonaires (GERM O P). Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. *Eur Respir J* 2005;26: 586–593.
13. Collum SD, Molina JG, Hanmandlu A, Bi W, Pedroza M, Mertens TCJ, *et al*. Adenosine and hyaluronan promote lung fibrosis and pulmonary hypertension in combined pulmonary fibrosis and emphysema. *Dis Model Mech* 2019;12:dmm038711.
14. Seike M, Ikeda M, Matsumoto M, Hamada R, Takeya M, Kodama H. Hyaluronan forms complexes with low density lipoprotein while also inducing foam cell infiltration in the dermis. *J Dermatol Sci* 2006;41: 197–204.
15. Principi N, Di Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. *J Transl Med* 2018;16:36.