Casting Iron in the Pathogenesis of Fibrotic Lung Disease

Clinical and experimental evidence highlights an important role for altered iron metabolism in the pathogenesis and severity of a number of lung diseases, including asthma, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF), and in increasing susceptibility to and worsening prognosis during respiratory infection (1–9) (reviewed in References 10 and 11). Although much of the literature describes links between altered systemic iron and lung disease, recent studies have demonstrated key roles for altered iron metabolism in the airways and lungs as playing important roles in the pathogenesis of disease (1–4, 6, 7).

Iron is increased in airway cells and tissues in clinical and experimental asthma and IPF, with increased iron levels correlating with worsened lung function in disease (1, 2). Increased iron accumulation in lungs of mice with genetic and dietary iron overload results in the development of lung pathology, including increased fibrosis and worsened lung function, even in the absence of experimental lung disease (1, 2, 12). Intriguingly, increased iron levels promote the proliferation and/or proinflammatory cytokine production and extracellular matrix gene expression in human lung fibroblasts and airway smooth muscle cells (1, 2). Although these findings suggest that increased iron accumulation in the lung may promote disease by increasing profibrotic and proinflammatory responses in fibroblasts and airway smooth muscle cells, evidence suggests that altered iron metabolism in other cells also plays key roles. Clinical and/or experimental studies show altered numbers of macrophages expressing differential levels of the iron uptake protein TFR1 (transferrin receptor 1) in the airway lumen or lung tissues in asthma and IPF (1–3). Importantly, studies show that $TFR1^+$ and $TFR1^-$ populations of macrophages are phenotypically and functionally distinct and have differential profibrotic and inflammatory gene expression profiles. This suggests that there are roles for altered TFR1-mediated iron uptake and metabolism in macrophages in the pathogenesis of disease. Iron accumulation has also been shown to be increased in sputum and lung tissue cells in clinical and experimental COPD (6, 7). Increased iron accumulation in airway mucosal cells in COPD results in oxidative stress–induced mitochondrial dysfunction, which plays a key role in the pathogenesis of disease (6). Iron metabolism in COPD is associated with decreased hepcidin responses both systemically and in the lung, which is associated with increased ferroportin on alveolar macrophages (AM) and decreased AM function (7).

In this issue of the *Journal*, Zhu and colleagues (pp. 189–200) describe a series of studies that used a combination of IPF patient samples, murine models of experimental IPF, and *in vitro* cell culture models to show how increased iron accumulation in lung tissues affects the pathogenesis of pulmonary fibrosis (13). The authors demonstrate significant iron accumulation, particularly in macrophages and monocytes, and increased ferritin light chain expression in fibroblasts in lung tissues of patients with IPF compared with controls. Using an elegant combination of immunofluorescence staining of lung sections and flow cytometric techniques, the authors show increased ferritin light and heavy chain protein accumulation and increased iron accumulation in both fibroblasts ($PDGFR\alpha^+$ cells) and macrophages ($CD68^+$ cells) in lung tissue in experimental IPF.

The authors then show that treatment with clioquinol (CQ), an antimicrobial agent with unique iron chelating properties, suppresses the increased iron accumulation that occurs in cells and in lung tissue during experimental IPF. Importantly, treatment-associated reductions in pulmonary iron accumulation are associated with a decrease in collagen I gene expression as well as collagen content, assessed using the hydroxyproline assay, and fibrosis, assessed using Masson's trichrome staining. CQ treatment also protected against changes in the expression of key iron-related factors induced during experimental IPF. The authors next used a complementary combination of *in vitro*, *in vivo*, and *ex vivo* studies to provide evidence that CQ mediates its protective effects during experimental IPF by preventing increased iron accumulation in fibroblasts. CQ treatment of fibroblasts *in vitro* results in a dose-dependent decrease in cytosolic labile iron, assessed by the calcein–AM flow cytometry method. Furthermore, CQ treatment reduces the number of total $PDGFR\alpha^+$ and proliferating $PDGFR\alpha^+/BRDU^+$ fibroblasts in the lungs during experimental IPF. They also show that CQ, as well as the iron chelating agents deferoxamine and deferiprone, suppress *ex vivo* proliferation of fibroblasts sourced from the lungs of mice with experimental IPF, whereas increasing iron levels using ferrous sulfate increases proliferation *ex vivo*. Additional studies also demonstrate that CQ reduces the migration of, and proinflammatory cytokine responses in, fibroblasts *ex vivo*. Interestingly, the authors show that media from macrophages treated with CQ, but not media from untreated macrophages, reduces the *ex vivo* proliferation of fibroblasts from mice with experimental IPF, suggesting that CQ may also mediate its antifibrotic effects in experimental IPF by modulating macrophage responses.

Together, the findings by Zhu and colleagues (13) and others (1–4, 6, 7) show critical roles for dysregulated iron metabolism in altering the function and phenotype of different cell populations in the airways and lung tissue and that dysregulated iron metabolism in these cells plays important roles in the pathogenesis of respiratory disease. Importantly, these studies highlight the therapeutic potential of correcting dysregulated iron metabolism for the treatment of IPF and other respiratory diseases. In terms of iron chelating agents, oral deferiprone has been shown previously to protect against experimental cigarette smoke–induced COPD (6), and treatment with zinc- and gallium-complexed deferoxamine protects against ovalbumin-induced experimental asthma (14). Iron chelators may also prove effective in reducing infection and improving lung function and prognosis in patients with CF, especially in combination with antibiotics, although

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studies are needed to translate promising *in vivo* and *in vitro* findings into effective therapies for patients with CF (15) as well as other lung diseases. Further research is also required to better understand how altered iron uptake and storage, especially through altered TFR1, hepcidin, ferroportin, and other iron-related protein responses, affect metabolism, phenotype, and function in different cell populations, both systemically and in the lungs, in different respiratory diseases. Such studies may inform new therapeutic strategies that target dysregulated iron metabolism, which is increasingly recognized as playing a crucial role in the pathogenesis and severity of respiratory disease. ■

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