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Ironing Out the Roles of Macrophages in Idiopathic Pulmonary Fibrosis

Airway macrophages (AMs) act to remove inhaled particles, debris, allergens, and microbes, making them crucial to host defense and epithelial homeostasis (1). Cross-talk between AMs, dendritic cells, alveolar epithelial cells, and T cells regulates how the immune system responds to environmental lung stimuli. Thus, AMs are pivotal in the response to alveolar injury and the subsequent biological pathways regulating resolution or persistence of alveolar inflammation (2). Given this keystone role, AMs have been studied in both human disease and animal models of pulmonary fibrosis (3–5), where the prevailing notion is that AMs derived from circulating monocytes worsen disease (5, 6). However, the mechanism(s) responsible for the ability of AMs to promote pulmonary fibrogenesis are unclear.

In this issue of the Journal (pp. 209–219), Allden and colleagues advance knowledge about macrophages and idiopathic pulmonary fibrosis (IPF) (7). They used BAL acquired from two independent IPF patient cohorts from observational clinical studies and interrogated specific lung leukocyte phenotypes by complimentary techniques, including multiparametric flow cytometry matched with immunohistochemistry. They show increased proportions of AMs lacking surface CD71 (the transferrin receptor) in human patients with IPF. The authors carefully interrogated the phenotype of these CD71⁻ AMs. Remarkably, CD71⁻ AMs demonstrated impaired function with defective phagocytosis, reduced markers of macrophage maturity, and profibrotic gene activation. In a further provocative turn, the authors demonstrated an association between reduced survival and higher proportions of CD71⁻ AMs in the BAL using a cohort of 97 patients with IPF. They conclude that CD71⁻ AM percentages may serve as a novel biomarker of IPF disease progression and that the CD71 pathway may represent a target for therapeutic manipulation.

CD71 is an integral membrane protein that binds diferric transferrin complexes to mediate uptake into the cell via receptor-mediated endocytosis. The majority of iron in circulation in the steady state is bound to transferrin. Transferrin–iron complexes bind CD71, which serves as a cellular receptor but also serves to limit the ability of iron to catalyze the formation of free radicals from reactive oxygen species, resulting in iron toxicity (8). Furthermore, control of free iron is an important host defense function because it limits availability of iron to potentially pathogenic bacteria in vivo.

Given these associations between macrophages, iron, bacteria, and fibrosis, it is interesting to speculate about how and why loss of CD71 on IPF macrophages may be important for disease pathogenesis. Previous studies have shown elevated levels of iron and altered iron metabolism in IPF lungs (9–11). Allden and

colleagues also demonstrated higher levels of transferrin, although they could not measure free iron, in the BAL of patients with IPF compared with control subjects.

Although there is an intriguing correlation among the patients with IPF themselves which shows the highest levels of BAL transferrin in patients with the lowest percentages of CD71$^+$ AMs, the fact that overall there were still more CD71$^+$ AMs in patients with IPF than in control subjects (in whom BAL transferrin levels are low) suggests that mechanisms in addition to CD71$^+$ AM clearance (e.g., vascular leak and synthesis by other cells) may regulate the levels of BAL iron/transferrin.

How might the abundance of transferrin in the BAL of patients with IPF promote disease, and how is this related to CD71 expression by AMs? One intriguing possibility is that transferrin-bound iron in lungs of patients with IPF may promote lung microbiome alterations or dysbiosis.

Interestingly, transferrin-bound iron is a growth factor for *Staphylococcus aureus* (12). In IPF, studies have shown that increased lung bacterial load (16S ribosomal DNA gene copies) predicts patients who will have rapidly progressive disease (13, 14). Furthermore, *Streptococcus* and *Staphylococcus* species were identified as key taxa found in patients with IPF exhibiting progressive disease (15). Thus, it is tempting to speculate that low levels of AM CD71 may promote pathobiont growth of *S. aureus* via increased availability of iron–transferrin complexes in the airspaces.

Another equally interesting hypothesis is that CD71$^-$ AMs showed reduced phagocytosis. Certainly, a defect in bacterial clearance by these AMs may promote increased bacterial load noted in IPF progression. How CD71 and/or iron–transferrin complexes might regulate phagocytosis is unknown. This could be via direct signaling in the AMs or via regulation of cellular homeostasis and metabolism. Additional studies are needed to determine whether loss of CD71 on AMs is playing a pathogenic role via regulation of phagocytosis or microbiota directly or whether loss of CD71 is merely a passive biomarker of the progressive disease environment. Although the authors have validated the loss of CD71 on AMs in two different IPF patient cohorts, this measurement requires BAL sampling, which limits implementation of this as a biomarker for clinical use. Recent guideline recommendations for diagnosis of IPF may reduce the number of BALs performed for diagnostic purposes in patients with IPF.

A final possibility is that loss of CD71 identifies a profibrotic macrophage phenotype. Of note, the CD71-deficient AMs expressed higher levels of MMP2 (matrix metalloproteinase 2), MMP8, vascular endothelial growth factor, and plasminogen activation inhibitor 1, which might lead to increased activation of TGFβ (transforming growth factor-β), increased angiogenesis, decreased matrix degradation, and increased deposition of extracellular matrix. Again, how CD71 signaling may mediate these effects is unclear. Expression of CD71 has been linked with tumorigenic proliferation in esophageal squamous cell carcinoma (16); thus, it may have proliferative effects in AMs also.

Potentially, CD71$^+$ AMs may expand and have a regulatory role in some patients. Certainly, recent single-cell transcriptomic analyses of human IPF lungs have revealed macrophage heterogeneity that is incompletely understood (17). It is also possible that low levels of CD71 characterize immature, recently recruited, monocyte-derived

AMs, consistent with murine studies suggesting that monocyte-derived AMs persist and promote lung fibrosis (6).

In conclusion, the study by Allden and colleagues provides another intriguing analysis of the role of macrophages in IPF. This study highlights correlations between disease progression, impaired innate immune function, and profibrotic mediator production and hints that these features may reside in inflammatory or recruited lung AMs. Thus, what's old is new again, and the field is rediscovering the likely importance of inflammatory/recruited AMs in the pathogenesis of IPF. Perhaps the finding that CD71 loss marks a potentially pathogenic subset of AMs will help to eventually "iron" out the role of this cell type in disease progression. ∎

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Selective Regulation of the Airway Mucin MUC5B in the Distal Airway

A common gain-of-function promoter polymorphism rs35705950 (G major allele, T minor allele) in the airway mucin MUC5B is the strongest and most replicated genetic risk factor for idiopathic pulmonary fibrosis (IPF) (1). It has been demonstrated that the *MUC5B* promoter variant affects MUC5B expression in the distal airways in IPF (2), and that Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice (3). However, less is known about the role this variant, located in a highly conserved region of the *MUC5B* promoter −3.5 kb upstream of the transcription start site (1), plays in transcriptional regulation of MUC5B. Publically available data through the Encyclopedia of DNA Elements (ENCODE) suggest this is a complex area of the genome with many transcription factors showing evidence of binding in the −3.5 kb region of the MUC5B promoter, in addition to the −0.1 kb proximal promoter (4). Another more general question in the field of mucin biology is that of selective mechanisms that differentially regulate MUC5B and its close neighbor and relative MUC5AC. Studies in recent years have shown that the transcription factors SPDEF (SAM pointed domain-containing ETS transcription factor) (5), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) (6, 7), and FOXA2 (Forkhead box protein A2) (4, 8) bind to both MUC5B and MUC5AC promoters, regulate their gene expression, and hence, lack the specificity needed to differentially regulate these two mucins (although FOXA3 is known to regulate MUC5AC specifically in Th2-dependent manner).

In this issue of the *Journal*, Chen and colleagues (pp. 220–234) identified a novel pathway that selectively regulates MUC5B, but not MUC5AC, expression in the distal airways (9). In their elegant and comprehensive report, they demonstrated three important findings. First, the endoplasmic reticulum to nucleus signaling 2 (ERN2) selectively promotes expression of the MUC5B mucin in distal airways via its downstream effector, the spliced form of the XBP1S (X-box–binding protein 1) transcription factor. In a series of meticulous experiments, the authors used human IPF tissue, *in vivo* animal models, and primary cells to elucidate the role of the spliced form of XBP1 in regulation of MUC5B, but not MUC5AC, expression in response to stimulation with cytokine IL1β. Among other findings, they show that there is a strong correlation of XBP1S and MUC5B mRNA on IL1β treatment, but not at baseline, whereas correlation of MUC5AC and XBP1S is weak at baseline and after treatment with IL1β. Second, XBP1S differentially regulates MUC5B promoter variant activity. Chen and colleagues report that induction of MUC5B(T) by XBP1S is greater than MUC5B(G) at all times tested by luciferase reporter activity. Finally, importantly, they also showed that pharmacologic inhibition and genetic deletion of ERN2-XBP1S reduced MUC5B expression. Inhibiting the ERN kinase had a moderate inhibitory effect, and deletion of XBP1 had a strong MUC5B inhibitory effect on expression levels. Higher levels of ERN2 and XBP1S were also observed in patients with IPF, and the results open potential avenues for novel therapeutic strategies using these observations. Using all data they collected, Chen and colleagues propose a "bistable model," which is a positive feedback loop by ERN2-XBP1S that explains accumulation of mucus in IPF (Figure 1). This model exhibits both a reversible state (low stimulus) and an irreversible state (high stimulus). In response to insults that produce injury/or inflammation that accelerates MUC5B transcription, ER stress is induced, ERN2 is activated, and spliced XBP1 increases UPR gene and MUC5B transcription rates. This response is reversible on removal of the injury/cytokine stimulus. However, the presence of the MUC5B promoter minor allele amplifies XBP1S-induced MUC5B transcription, producing an irreversible positive feedback state that may be sufficient to trigger impaired host defense and accelerate cell senescence and/or damage.

The report by Chen and colleagues is a major step forward in understanding the selective regulation of MUC5B expression levels