C(C)Learing the Role of Chemokines in Pulmonary Fibrosis

Pulmonary fibrosis (PF) is a class of diseases with aberrant scarring of the lung tissue that leads to progressive loss of function. The most common form, idiopathic PF (IPF), has a high mortality and limited treatment options. Historically, PF was thought of as a chronic inflammation–driven condition, as multiple proinflammatory cytokines and chemokines are found to be elevated in subjects with PF and in experimental animal models (1). However, the PANTHER (Prednisone, Azathioprine, and *N*-acetylcysteine) trial, which used antiinflammatory and immunosuppressive medications to treat IPF, failed to improve physiological parameters, and in fact led to increased mortality and hospitalizations in the treatment group (2). Clearly, there are still many gaps in our knowledge regarding the observed elevated inflammatory markers, their biological properties, and their potential as therapeutic targets.

CCL2 and its receptor, CCR2, are among the most extensively studied pairs of chemokines and receptors in PF. Elevated CCL2 has been found in BAL fluid and serum samples from subjects with IPF (3, 4). Moreover, alveolar epithelial cells (AECs) within fibrotic areas were reported to have augmented CCL2 expression in subjects with IPF (5). In animal models of PF, CCL12, the analog of CCL2 in humans (6), was found to be elevated in lung tissue of mice developing fibrosis, and $CCR2^{-/-}$ mice were shown to be protected from developing PF (3, 7). The data on $CCL2^{-/-}$ mice are inconclusive, as Baran and colleagues found that $CCL2^{-/-}$ mice were protected when they used a peritoneal bleomycin administration model, but in an FITC intratracheal installation model the $CCL2^{-/-}$ mice were not protected (3, 8). The biological relevance of the increased levels of CCL2 has been proposed to arise from its ability to recruit a group of profibrotic cells, termed fibrocytes, that express CD45 and mesenchymal collagen I (9). Despite the abundant observational and preclinical data, a clinical trial using a monoclonal antibody to CCL2 failed to show any protective effects of neutralizing CCL2 in subjects with IPF, and was stopped prematurely owing to a greater loss in forced vital capacity in the treatment group, although the overall mortality was not different compared with placebo (10). Unexpectedly, subjects who received the CCL2 monoclonal antibody actually had higher total and free CCL2 in their serum than the placebo-treated subjects. This paradoxical observation gave rise to a theory that a compensatory mechanism may exist in the presence of CCL2 blockade. In addition, global CCL2 blockade could be deleterious, as CCL2 might work in other important antifibrotic pathways that remain to be determined. A recent paper showed that one target of CCL2 is $CCR2^{+}CD4^{+}$ T cells (11). These T cells function similarly to regulatory T cells and were found to exert an antifibrotic effect in an experimental animal model.

In a study presented in this issue of the *Journal*, Yang and colleagues (pp. 622–632) generated both CCL12 global knockout mice and a conditional CCL12 deletion in AECs (12). The authors compared CCR2 ligand expression in these mice using a bleomycin-injury model. They found that CCL12 global knockout in mice led to a compensatory elevation of CCL2 and CCL7 in lung tissue and BAL fluid compared with wild-type mice. Furthermore, the CCL12 global knockout mice were not protected from developing fibrosis, suggesting that other CCR2 ligands have an equally important role in fibrosis. In contrast, the conditional AEC CCL12 knockout mice (driven by SPC-rtTA/tetO-Cre promoter) were protected. CCL2 and CCL7 levels in lung tissue and BAL fluid did not differ from those observed in control mice after bleomycin injury. Because CCL2 is known to be highly expressed in AECs of subjects with IPF, these data suggest that CCL12 expression plays an important role during fibrogenesis, and that the selective deletion of CCL12 in AEC may serve as a potential target for intervention.

Mechanistically, the authors propose that AEC-derived CCL12 recruits exudate macrophages, and deletion of CCL12 leads to reduced exudative macrophage recruitment to the lung after bleomycin injury. In addition, CCL2 and CCL12 activated mTOR, and inhibition of mTOR with a chemical inhibitor (torin) or silencing of an mTORC1 component, raptor, reduced CCL2 and CCL12 production in epithelial cells; however, the involvement of mTOR was not determined *in vivo*. A previous study in $CCL2^{-/-}$ mice showed a reduced number of macrophages after bleomycin administration (3). Given recent studies demonstrating that monocyte-derived macrophages promote fibrosis progression (7, 13, 14), it is not surprising that exudate macrophages (defined in this article as $SiglecF^{-}$ $CD11c^{+}$autofluorescent^{+}) were reduced in mice harboring a conditional deletion of CCL12 in AECs.

Although the study by Yang and colleagues provides new evidence that conditional knockout of CCL12 in AECs can protect mice from PF, many questions remain unanswered. The biological properties of exudate macrophages need to be further defined by gene expression profiling or transcriptome analysis to verify that these cells are indeed profibrotic. It has been reported that mTORC1 regulates CCL2 production by activating the transcription factor FOXK1 in HeLa cells, and increasing CCL2 production leads to augmented recruitment of tumor-associated macrophages, which share many similarities with profibrotic macrophages (15). Although the data presented by Yang and colleagues show inhibition of the mTOR pathway with torin (which inhibits both mTORC1 and mTORC2) or raptor shRNA (mTORC1 complex), it is unclear whether mTORC2 (rictor) also contributes to the

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regulation of CCL2 production in AECs. The PI3K/Akt/mTOR pathway is an important regulator of cell senescence, which is a cardinal feature of IPF (16). It is unclear whether changes in CCL2 production have any effects on cell senescence, proliferation, or survival. AECs from $CCR2^{-/-}$ mice exposed to bleomycin have increased caspase 3 activity (7); however, it is critical to determine whether the conditional deletion of CCL12 in AECs attenuates apoptosis, as these mice are protected from PF.

In this study, Yang and colleagues are the first to generate complete and AEC-specific CCL12 knockout mice and to characterize their role in PF. Their transgenic mice have the potential to become a powerful tool in future research assessing the role of chemokines in PF and whether we can target this pathway for drug development. This article also highlights a new population of cells, exudate macrophages, that CCL12 targets to provide additional information about the vital role of macrophages in fibrogenesis. ■

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References

1. Agostini C, Gurrieri C. Chemokine/cytokine cocktail in idiopathic pulmonary fibrosis. *Proc Am Thorac Soc* 2006;3:357–363.
2. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ; Idiopathic Pulmonary Fibrosis Clinical Research Network. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *N Engl J Med* 2012;366:1968–1977.
3. Baran CP, Opalek JM, McMaken S, Newland CA, O'Brien JM Jr, Hunter MG, *et al*. Important roles for macrophage colony-stimulating factor, CC chemokine ligand 2, and mononuclear phagocytes in the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med* 2007; 176:78–89.
4. Suga M, Iyonaga K, Ichiyasu H, Saita N, Yamasaki H, Ando M. Clinical significance of MCP-1 levels in BALF and serum in patients with interstitial lung diseases. *Eur Respir J* 1999;14:376–382.
5. Mercer PF, Johns RH, Scotton CJ, Krupiczojc MA, Königshoff M, Howell DC, *et al*. Pulmonary epithelium is a prominent source of proteinase-activated receptor-1-inducible CCL2 in pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:414–425.
6. Sarafi MN, Garcia-Zepeda EA, MacLean JA, Charo IF, Luster AD. Murine monocyte chemoattractant protein (MCP)-5: a novel CC chemokine that is a structural and functional homologue of human MCP-1. *J Exp Med* 1997;185:99–109.
7. Larson-Casey JL, Vaid M, Gu L, He C, Cai GQ, Ding Q, *et al*. Increased flux through the mevalonate pathway mediates fibrotic repair without injury. *J Clin Invest* 2019;129:4962–4978.
8. Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB. The role of CCL12 in the recruitment of fibrocytes and lung fibrosis. *Am J Respir Cell Mol Biol* 2006;35:175–181.
9. Moore BB, Kolodsick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C, *et al*. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *Am J Pathol* 2005;166:675–684.
10. Raghu G, Martinez FJ, Brown KK, Costabel U, Cottin V, Wells AU, *et al*. CC-chemokine ligand 2 inhibition in idiopathic pulmonary fibrosis: a phase 2 trial of carlumab. *Eur Respir J* 2015;46:1740–1750.
11. Milger K, Yu Y, Brudy E, Irmler M, Skapenko A, Mayinger M, *et al*. Pulmonary $CCR2^{+}CD4^{+}$ T cells are immune regulatory and attenuate lung fibrosis development. *Thorax* 2017;72:1007–1020.
12. Yang J, Agarwal M, Ling S, Teitz-Tennenbaum S, Zemans RL, Osterholzer JJ, *et al*. Diverse injury pathways induce alveolar epithelial cell CCL2/12, which promotes lung fibrosis. *Am J Respir Cell Mol Biol* 2020;62:622–632.
13. He C, Larson-Casey JL, Davis D, Hanumanthu VS, Longhini ALF, Thannickal VJ, *et al*. NOX4 modulates macrophage phenotype and mitochondrial biogenesis in asbestosis. *JCI Insight* 2019;4: 126551.
14. Misharin AV, Morales-Nebreda L, Reyfman PA, Cuda CM, Walter JM, McQuattie-Pimentel AC, *et al*. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J Exp Med* 2017;214:2387–2404.
15. Nakatsumi H, Matsumoto M, Nakayama KI. Noncanonical pathway for regulation of CCL2 expression by an mTORC1-FOXK1 axis promotes recruitment of tumor-associated macrophages. *Cell Rep.* 2017;21:2471–2486.
16. Lawrence J, Nho R. The role of the mammalian target of rapamycin (mTOR) in pulmonary fibrosis. *Int J Mol Sci* 2018;19:E778.