CCL2 and T cells in pulmonary fibrosis: an old player gets a new role

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Idiopathic pulmonary fibrosis (IPF) is an incurable condition characterised by progressive extracellular matrix deposition and tissue remodelling in the adult human lung.[1] Because 5-year mortality rates approach 70%, new approaches to treatment are sorely needed. IPF is thought to result from an abnormal wound healing response caused by an unknown insult to the lung epithelium that results in the recruitment and activation of myofibroblasts via incompletely understood mechanisms.[1] The contribution of immune activation to these processes remains unknown. While the contribution of innate cell populations such as macrophages is gaining increased acceptance, the contribution of T cell driven adaptive immune responses remain controversial.[2] The paper by Milger *et al*[3] represents an important step forward in our understanding of this issue.

Historically, IPF has been defined as a non-immune entity due to its lack of an identifiable antigen driven immune response, lack of inflammatory infiltrate on lung biopsy, lack of benefit from conventional immunosuppressive therapies and lack of a requirement for lymphocytes for development of maximal fibrosis in commonly used animal models.[1 2] However, the now seminal finding that lymphocyte modulating agents actually worsen outcomes in IPF[4] combined with human and animal data demonstrating an imbalance between T helper populations in these settings suggests a critical role for lymphocytes in the orchestration of fibrotic responses. For instance, Th2 and Th17 cells are associated with fibrogenesis, while Th1, Th22 and γδ-T cells appear to be at least partially protective.[5–9] In contrast, regulatory T cells (Tregs) can differentially promote or suppress fibrosis depending on the setting.[10] When viewed in combination, these data suggest a cell-specific and state-specific contribution of T lymphocytes to IPF, leading to the growing recognition of T cell heterogeneity as an important area of investigation for this incurable disease.

CCL2 is a chemokine which orchestrates immune cell recruitment via its preferential binding to the CCR2 receptor.[11] It is induced during inflammatory responses when immune cells are required for tissue repair.[11] Both human and murine studies have shown that CCL2 contributes to fibrosis through a variety of mechanisms involving inflammation, angiogenesis and myofibroblast accumulation.[12] Specifically, elevations in CCL2 have been found in the serum,[13] bronchoalveolar lavage[14] and the alveolar epithelium[15] of patients with IPF, and deletion or inhibition of CCR2 reduces pulmonary fibrosis in several experimental settings.[16] Furthermore, studies using murine models of pulmonary fibrosis and primary IPF lung fibroblasts suggest an interplay between TGFβ1, interleukin (IL)13 and CCL2 promotes fibroproliferation[17 18] and excessive activation of the CCR2/CCL2 axis is predictive of poor prognosis in patients with IPF.[19]

Based on this preclinical evidence, Carlumab (a humanised monoclonal antibody targeting CCL2) was tested for efficacy in a randomised controlled trial of patients with IPF.[12] This study, which was published in 2015, failed to find a benefit for Carlumab therapy and in fact was terminated early due to a trend towards worsening of lung function in one of the treatment arms. Now, for the first time, the study by Milger *et al*[3] offers a possible explanation to the biology behind these observations.

Milger *et al*[3] identify CCR2 expressing CD4+ T lymphocytes as a subset of CD4+ cells displaying anti-inflammatory and antifibrotic properties and explore their role in lung fibrosis. Specifically, studies performed in the bleomycin mouse model reveal the accumulation of CCR2 expressing CD4+ T cells (CCR2+ CD4+) in the fibrotic lung. The majority of these cells were phenotyped as effector memory T cells and demonstrated migratory potential due to their expression of homing receptors. Curiously, however, these cells also bear hallmarks of natural Tregs in that they express high levels of FoxP3 and IL-10. Functionally, CCR2+ CD4+ cells suppress effector T cell proliferation in similar magnitude as natural Tregs and adoptive transfer of these cells improves fibrosis in bleomycin treated mice. In human correlates of these findings, increased concentrations of CCR2+CD4+ cells are seen in bronchoalveolar lavage specimens from patients with IPF when compared with controls. Taken together, these data show CCL2 might act on these CCR2+ CD4+ cells to abrogate inflammation and fibrosis, and suggest that the therapeutic failure of Carlumab might be related to suppression of this population.

The impact of the study by Milger *et al*[3] cannot be overstated. In addition to offering a potential explanation for the surprising failure of CCL2 targeted therapies, the work provides more global insight into IPF. For example, it supports the emerging concept that a single receptor such as CCR2 might play different roles depending on its temporospatial expression during injury and repair. It also suggests that CCL2 might play equal and opposing roles in innate and adaptive responses; specifically, to function as a recruiter of fibrosis-promoting macrophages while simultaneously inducing the accumulation of immunosuppressive Treg population. However, because CCR2 is also expressed on alveolar epithelium and fibroblasts, the effects of CCL2 neutralisation might extend to the regulation of injury and repair responses in stromal cells. It also demonstrates the need for improved modelling systems since the function of the CCR2+ CD4+ cells in the context of experimentally induced fibrosis in mice may not necessarily recapitulate the situation in human disease. The study also highlights the need for personalised and specific treatments that can selectively target detrimental cells of interest and supports the growing consensus that in the complex milieu of the fibrotic lung, interruption of a single receptor-ligand interaction may be insufficient to overcome the activation of multiple competing and compensatory pathways.

As with any novel finding, many questions remain. The relationship of these CCR2+ CD4+ cells to other lymphocyte populations, as well as to other immune cells present in the IPF lung, will require further investigation. The contribution of these cells to injury and remodelling remains undeciphered, though the generation of mice with inducible CCR2 deletion specifically targeted to CD4+ cells might answer this question. The relationship of CCR2+ CD4+ cells to the IPF disease state remains unelucidated, as does their relationship with disease progression

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and response to therapy. Nevertheless, the study by Milger *et al*[3] firmly frames CCR2+ CD4+ cells as a new and exciting area of study of CCL2 biology as it relates to pulmonary fibrosis. Further understanding of these findings will advance the evolving understanding of the complex contribution of lymphocyte heterogeneity to IPF and related conditions affecting the adult mammalian lung.

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