Title: Targeting recruitment of CCR2+ monocytes protects CF mice from TGFβ-driven lung tissue damage following chronic inflammation

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Background: Cfttm1Unc (CFKO) mice chronically nebulized with lipopolysaccharide (LPS) recapitulate several features of late-stage cystic fibrosis (CF) lung disease. They have increased numbers of recruited, C-C Motif Chemokine Receptor 2 (CCR2) expressing monocytes/macrophages, neutrophilia, as well as elevated transforming growth-factor β (TGFβ) levels in bronchoalveolar lavage fluid (BALF) and TGFβ signaling in lung tissues. Pathologic TGFβ signaling leads to collagen deposition, lung remodeling, and ultimately irreversible tissue damage. CCR2−/− mice, which have impaired recruitment of inflammatory monocytes (iMons) have lower TGFβ levels and signaling and are protected from lung tissue damage.

Aims: To assess whether blocking iMon recruitment ameliorates TGFβ levels and protects CF mice from lung tissue damage.

Methods: To do so, we used CCR2−/− x CFKO double knockout mice (dKO; n=23) as well as CCR2 inhibitor (RS 102895, Tocris) treated CFKO mice (n=6). Mice were nebulized with 12.5 mg LPS (from P. aeruginosa) three times a week for 5 weeks (chronic LPS). WT, CFKO and CCR2−/− mice were used as controls. Lung immune cells (iMons, interstitial MΦs (IMs), monocyte-derived (mo) and tissue-resident alveolar MΦs (trAMs), neutrophils, T and B cells) were assessed by flow cytometry. TGF-β levels in BALF (ELISA), TGF-β signaling (qPCR, western blot), collagen depositions (trichrome stained sections), lung pathology scores and weight loss were assessed.

Results: CCR2 deficiency in a CF background (dKO mice) significantly reduced recruited macrophage populations in lungs after chronic LPS to levels similar to CCR2−/− mice, which also ameliorated lung neutrophil infiltration as well as T and B cell numbers and weight loss to levels comparable to WT mice. The lack of recruited macrophages also correlated with decreased TGFβ levels and signaling, and lowered collagen depositions and alveolar tissue damage. Pharmacological inhibition of CCR2 lowered numbers of AMs (moAMs and trAMs), IMs, iMons, neutrophils, DCs, CD4 and CD8 T cells, and B cells as well as TGFβ levels in BALF and TGFβ signaling in lung tissues to levels comparable to WT mice following chronic LPS.

Conclusion: Our data indicates that pharmacological inhibition of CCR2 might serve as a therapeutic intervention to prevent lung tissue remodeling in CF.

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