Title: Macrophages of monocytic origin drive pathological TGF-β levels and tissue remodeling in chronically inflamed CF lungs

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Background: TGF-β levels are elevated in bronchoalveolar lavage fluid (BALF) of patients with Cystic Fibrosis (CF), leading to collagen deposition, lung remodeling, and, ultimately irreversible tissue damage. Cftrtm1Unc (CFTR) mice chronically nebulized with LPS recapitulate features of late-stage CF lung disease. Macrophages (MΦs), which are dysfunctional and increased in numbers in lungs of patients with CF, are key regulators of tissue homeostasis. Their role in CF lung remodeling remains elusive.

Aims: To study the dynamic changes of lung MΦ populations and their contribution to TGF-β signaling and lung remodeling during chronic inflammation in CF.

Methods: WT (n=30) and CFTR (n=24) mice were nebulized with 12.5 mg LPS (from P. aeruginosa) 3 times a week for 5 weeks (chronic), followed by 6 weeks of recovery time. CCR2−/− (CCR2, n=20) were used to determine the significance of CCR2+ inflammatory monocyte (iMon) recruitment. Lung immune cells (iMons, interstitial MΦs (IMs), monocyte-derived (MO-) and tissue-resident alveolar MΦs (TR-AMs) and neutrophils) were assessed by flow cytometry. TGF-β levels in BALF (ELISA), TGF-β signaling (qPCR, western blot) and collagen depositions (trichrome stained lung sections) in lung tissues were assessed. RNAseq was performed on sorted lung MΦ populations of WT and CFTR mice.

Results: Lung tissues of CFTR mice had significantly higher numbers of iMons, IMs, MO-AMs and neutrophils after chronic LPS compared to WT mice. This correlated with higher TGF-β levels, TGF-β signaling and collagen depositions in lungs of CFTR mice compared to WT. Impaired migration of iMons in CCR2 mice lead to lower numbers of iMons, IMs and MO-AMs after chronic LPS and significantly lower TGF-β levels and collagen depositions compared to WT and CFTR mice. Lungs of WT and CCR2 mice recovered from chronic inflammation, while CFTR mice had persistent lung tissue damage and pertained increased immune cells after recovery time. RNAseq analysis comparing WT to CFTR MΦ populations revealed that iMons have the most differentially expressed genes.

Conclusion: Our data indicates that monocyte-derived MΦs in CF mice lead to excessive TGF-β-driven lung remodeling. Furthermore, we see a failure to recover from chronic inflammation, which is possibly caused by altered expression profile of iMons. Supported by the Cystic Fibrosis Foundation (OZ18F0).