Defining the differences between acute and chronic lung fibrosis

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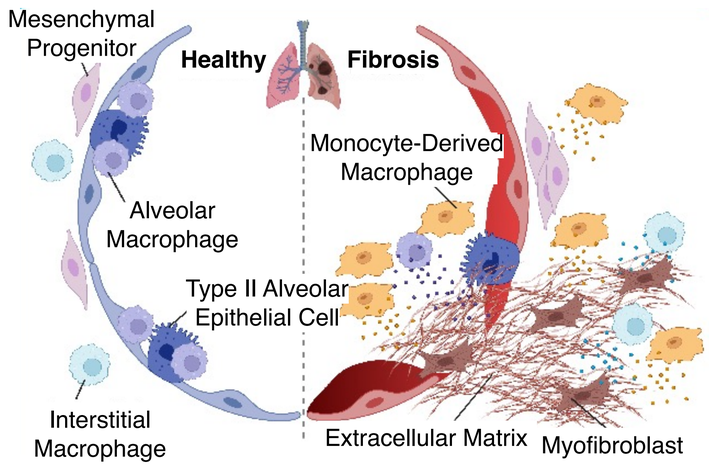
Introduction

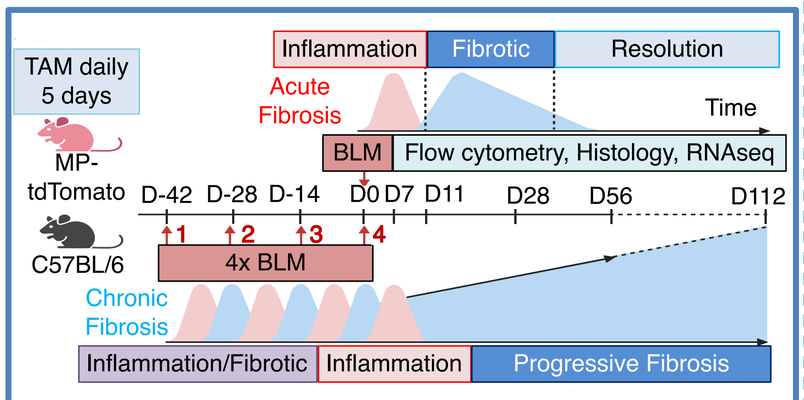
Pulmonary fibrosis (PF) is a chronic progressive and fatal disease with no cure for PF. A single dose of Bleomycin (BLM) is the commonly used for induction of experimental lung fibrosis, however, it can not mimic the progressive and irreversible features of human PF. Intratracheal administration of BLM results in injury and an acute inflammatory response is induced, followed by fibrotic changes resulting in deposition of extracellular matrix (ECM) in the lung. However, the fibrotic phase resolves, with loss of myofibroblasts and degradation of ECM. ***The goal of this study is to establish a chronic fibrosis model for PF through administration of multiple dose of BLM and to determine the differences between resolvable (acute) and progressive (chronic) fibrosis.***

Here, we compare the key immune populations between acute and chronic fibrosis over time. Macrophages are involved in all stages of fibrosis. Interstitial macrophages (IM) and alveolar macrophages (AM) are critical in regulating homeostasis and resolution by phagocytosis of myofibroblasts and degradation of ECM. Fibrotic macrophages, on the contrary, produce cytokines that promote the proliferation of myofibroblasts and induce ECM deposition. ***Thus, we hypothesize the regulation of fibroblasts by lung macrophages is dysregulated in chronic fibrosis, contributing to persistent fibrosis.***



Schematic diagram of healthy and fibrotic lungs.

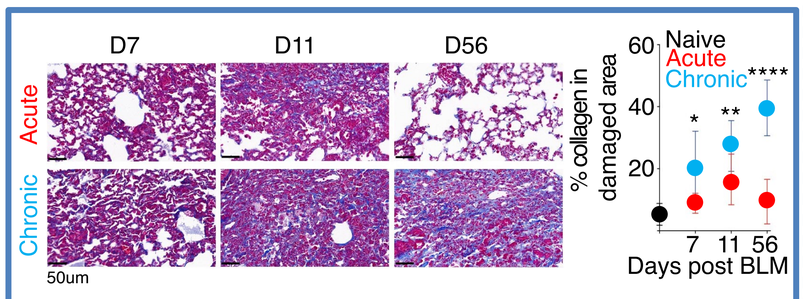
Methods



Experimental outline for acute and chronic fibrosis models. BLM: bleomycin; TAM: tamoxifen; MP: mesenchymal progenitor.

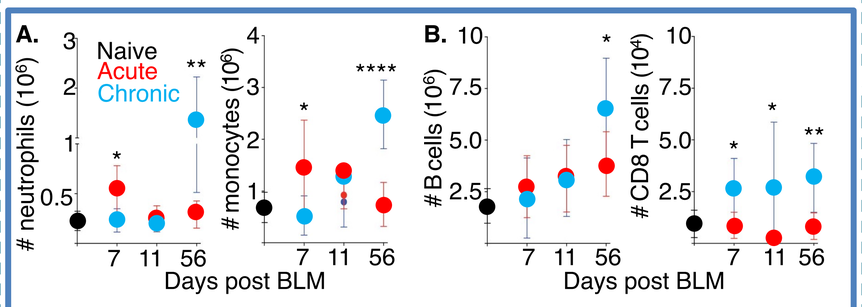
Results

1. Collagen deposition accumulates in chronic fibrosis over time.



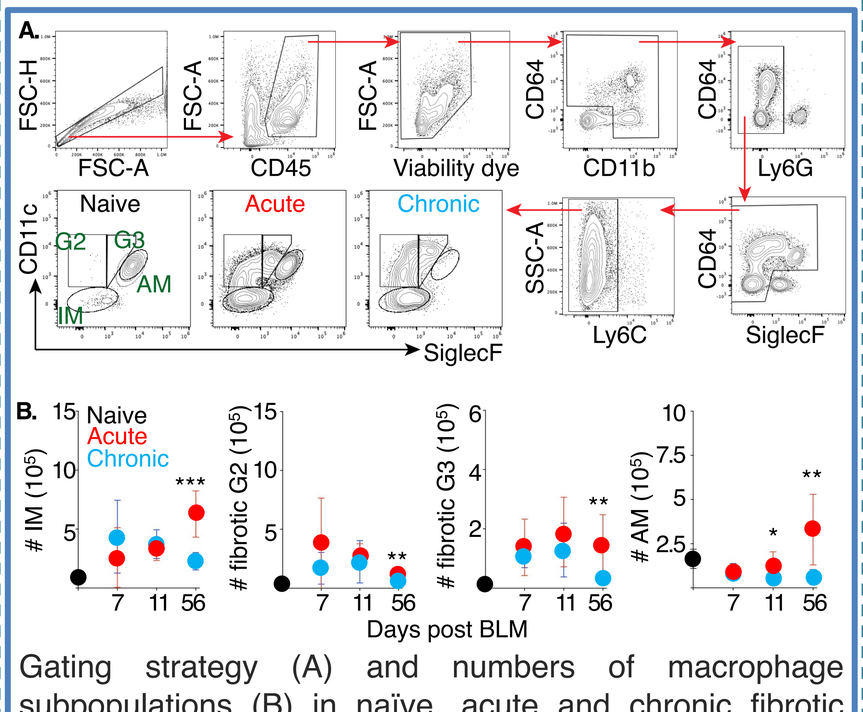
Representative images and quantification of lungs stained with Masson's Trichome without hematoxylin (blue staining for collagen). *p<0.05; **p<0.01; ****p<0.0001; unpaired Student t test.

2. Increased inflammatory innate & adaptive immune cells in chronic fibrosis, while acute fibrosis resolves.



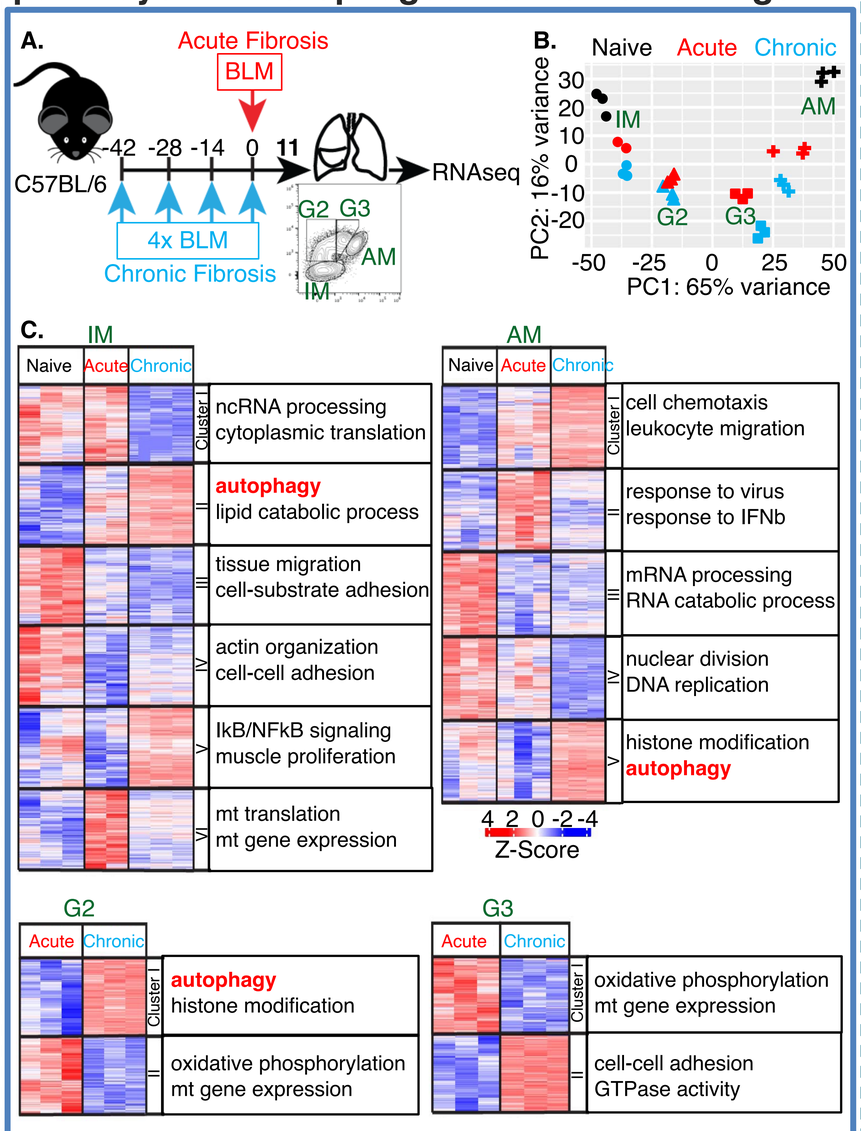
Numbers of innate (A) and adaptive (B) immune cells in naïve, acute and chronic fibrotic lungs over time after induction of injury by bleomycin (BLM). *p<0.05; **p<0.01; ****p<0.0001; unpaired Student t test.

3. Over time, lung macrophage populations decrease in chronic fibrosis but increase as acute fibrosis resolves.



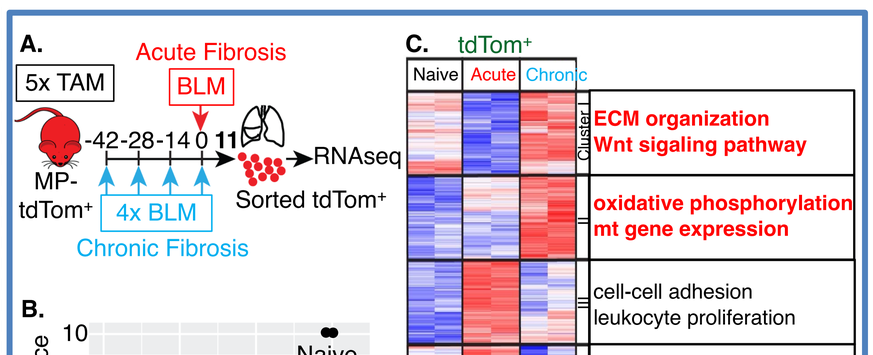
Gating strategy (A) and numbers of macrophage subpopulations (B) in naïve, acute and chronic fibrotic lungs over time. **p<0.01; ***p<0.001; unpaired Student t test.

4. Altered gene expression in autophagy Go pathways in macrophages from chronic lungs.



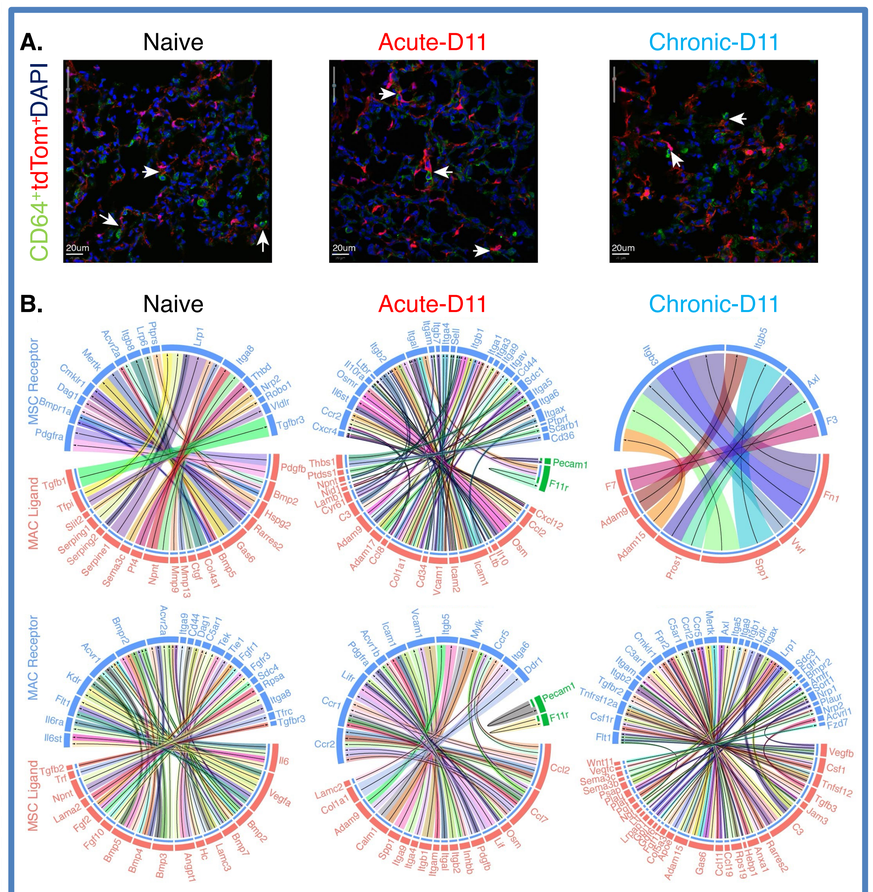
(A) Scheme of workflow to analyze RNAseq of macrophages from naïve, acute & chronic fibrotic lungs. Principal-component analysis (B) and k-means clustering of all identified genes (C) in sorted macrophages.

5. Upregulated gene expression in extracellular matrix & mitochondrial gene Go pathways in mesenchymal cells from chronic fibrosis.



(A) Scheme of workflow to analyze RNAseq of mesenchymal progenitors from naïve, acute & chronic fibrotic lungs. Principal-component analysis (B) and k-means clustering of all identified genes (C) in sorted mesenchymal progenitors.

6. While macrophage and mesenchymal cell remain in close proximity in all conditions, diverse ligand-receptor pairings between the two cell types are observed.



(A) Representative images showing proximate contact of macrophages (CD64$^+$) with mesenchymal cells (tdTom$^+$). (B) Circos plots ligand-receptor pairs showing key communication pathways used by macrophages and mesenchymal cells.

Summary

- We have established a mouse model for human PF by repetitive instillation of bleomycin.
- Significant differences between acute and chronic fibrosis:

a. Accumulated collagen deposition over time
b. Increased inflammatory immune cells but decreased macrophage populations in progression of chronic fibrosis
c. Upregulated gene expression in ECM & mitochondrial function in chronic mesenchymal cells
d. Upregulated gene expression associated with autophagy in chronic macrophages
e. Diverse ligand-receptor pairings are used by macrophage-mesenchymal cells in naïve, acute and chronic lungs

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