Modeling Macrophage-Fibroblast Interactions in Lung Regeneration

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ABSTRACT

In the lung injury repair process, alveolar macrophages localize near the injury site and have differential interactions with injury-associated fibroblasts and epithelial progenitors to induce injury repair. Alveolar macrophages and fibroblasts of injured lungs interact to respond to inflammation and fibrosis. To study the alveolar macrophage and its interaction with fibroblasts in depth, we have established an *in vitro* assay of alveolar macrophages (AM) collected through bronchoalveolar lavage (BAL) and compared them to bone marrow-derived macrophages (BMDM), a classical way to study macrophages *in vitro*. AM and BMDM were polarized to different subtypes of macrophages and were cocultured with the fibroblasts. RT-qPCR results have confirmed that both AM and BMDM can be polarized to different subtypes and macrophage-fibroblast coculture downregulates myofibroblast markers. The *in vitro* assay developed can be used to further study the macrophage's interaction with epithelial progenitors and the role of macrophage-fibroblast crosstalk during alveolar regeneration.

BACKGROUND

Macrophages are part of the innate immune system and serve an important role in first-line defense against pathogens and tissue injury. They are derived from the pluripotent hematopoietic stem cells in the bone marrow and mature into macrophages as they migrate into the tissues.[1] The mature macrophages that stay in the tissue are called the tissue-resident macrophages. They take an active role in maintaining homeostatic functions such as clearing the cell debris and dying cells. They also serve their immune function by responding to infection and resolving the inflammation[1]. In the case of injury or inflammation, the macrophages migrate to the site of injury and ingest the pathogens and cell debris through phagocytosis[2].

Depending on the injury and cytokines present at the injury site, macrophages can be polarized to M1 and M2, two distinct functional phenotypes of macrophages, that contribute to different responses. The classically activated macrophages (M1) are polarized with cytokines and chemokines such as TNF-α and IL-1β and produce a pro-inflammatory, tissue-damaging response. In contrast, alternatively activated macrophages (M2) can be divided into different subsets based on their function. They are polarized with cytokines IL-4, IL-13, IL-10, etc., and contribute to tissue repair, infection/pathogen clearance, and anti-inflammatory response[3] .

M1	M2a	M2b	M2c	M2d
Pro-inflammatory	Allergic reactions	Th2 response	Pro-fibrotic	Tumor-associated macrophages
LPS, TNF-α, IFN-γ	IL-4, IL-13	IL-1β, TLR ligands	IL-10, TGF-β	IL-6, TLR ligands

Table 1: Macrophage polarization states, phenotypes and activating cytokines[3].

As a barrier organ, the lung is exposed to infectious insults which can induce damage to the lung epithelium, especially in the pulmonary alveoli compartment. Alveolar macrophages are resident macrophages to the pulmonary alveoli and produce various signaling chemicals to regulate an immune response and tissue homeostasis[4]. Alveolar macrophages also interact with injury-associated fibroblasts and secrete pro-inflammatory cytokines to enhance local immune response as well as anti-inflammatory cytokines to resolve inflammation and produce fibrosis. Recent studies also indicate these innate immune cells may support epithelial cell proliferation and, thus, alveolar regeneration[5]. We have recently observed that alveolar macrophages and fibroblasts are associated with impaired epithelial regions and interact with epithelial progenitors in the alveolar regeneration process.

In our study, we aimed to study macrophage and fibroblast interactions *in vitro* by expanding on existing methodology with a focus on how macrophages could impart phenotypic changes in distinct fibroblasts. To establish this method, we compared bone marrow-derived macrophages (BMDM), a classical way to study macrophages *in vitro*, and alveolar macrophages (AM) collected through bronchoalveolar lavage (BAL). We also cocultured those macrophages with fibroblasts to study changes in gene expression and morphology.

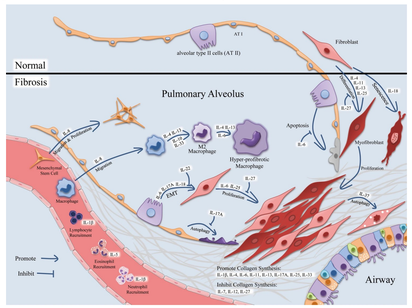

Illustration of the role of interleukin signaling in cell function and interactions in the alveolus during fibrotic lung injury[6].

METHODS

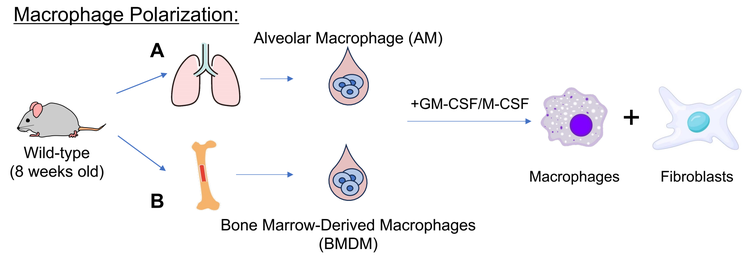

Figure 1: Collection and culture methods of two types of murine macrophages. (A) Alveolar macrophages were collected from the lung using BAL. 1 ml washes of BAL buffer (PBS + 2 mM EDTA) were flushed into the lung and extracted using a syringe. **(B)** Bone marrow was collected from femurs and tibiae by flushing PBS through the bone using a 25G needle. Granulocyte macrophage colony-stimulating factor (GM-CSF) produced by L929 cells was used to culture and promote macrophage growth. Both AMs and BMDMs were grown in the growth media (RPMI + 20% FBS + 30% L929 Sup + 1% P/S). For coculture experiments, fibroblasts were added to macrophage culture in a 1:2 ratio.

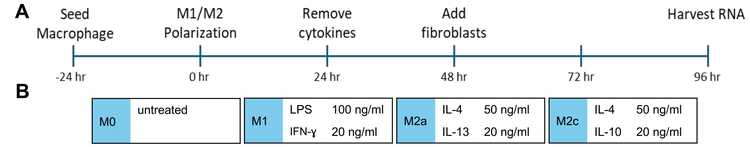

Figure 2: Experimental setup for polarization and coculture. (A) Timeline of experiment is shown. Macrophages were grown in replating media (RPMI + 10% FBS + 15% L929 Media + 1% P/S) 24 hours prior to polarization. Macrophages were exposed to cytokines for 24 hours in stimulation media (RPMI + 10% FBS + 5% L929 Media + 1% P/S) and RNA was harvested 48 hours after polarization. For macrophage-fibroblast coculture, fibroblasts were added to the macrophage culture 48 hours after polarization and were cultured for an additional 48 hours. **(B)** Table of cytokines and respective concentrations used for macrophage polarization.

RESULTS

Macrophages can be polarized to different subtypes *in vitro*

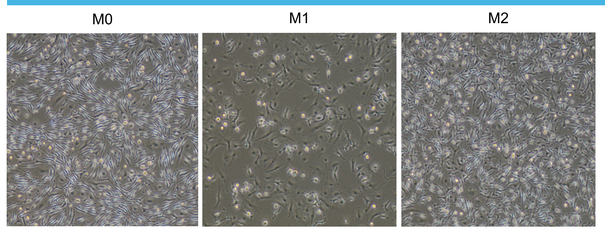

Figure 3: Polarized macrophages are morphologically distinct. Phase contrast images of BMDMs 48 hours after polarization. M1 macrophages are rounded, whereas M2 macrophages are more elongated and spindle-like.

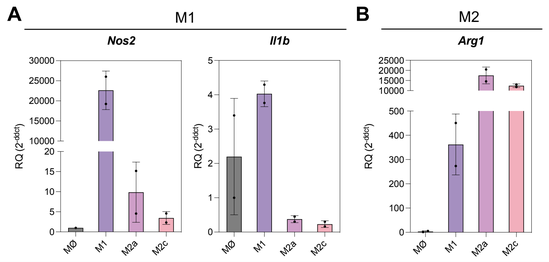

Figure 4: Hallmark genes of macrophage polarization are upregulated in their respective conditions. RT-qPCR results of BMDMs and AMs 48 hours after polarization. Polarization of BMDMs is validated by increased expression of **(A)** M1 markers *Nos2* and *Il1b* and **(B)** M2 marker *Arg1* in the respective macrophage phenotype. Fold change in relative gene expression measured using *Gapdh* as a reference.

RESULTS cont.

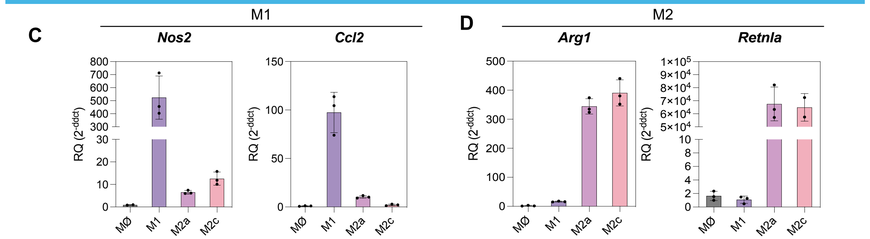

Figure 4 (cont.): Polarization of AMs similarly results in the expected expression of **(C)** M1 marker genes *Nos2* and *Ccl2* and **(D)** M2 marker *Arg1* and *Retnla* in the respective conditions.

Polarized macrophages exhibit distinctive behaviors in co-culture

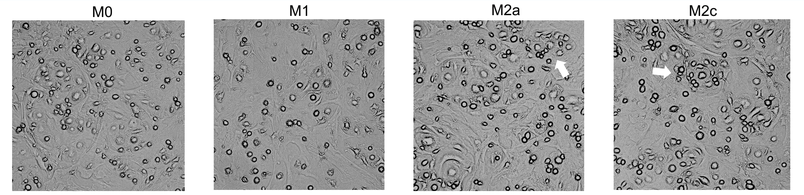

Figure 5: Differences in spatial arrangement of macrophages and fibroblasts. Phase contrast imaging of AMs cocultured with primary mouse lung fibroblasts after 48 hours. Unlike M1 AMs, M2a and M2c AMs cocultured with fibroblasts form clusters (white arrows).

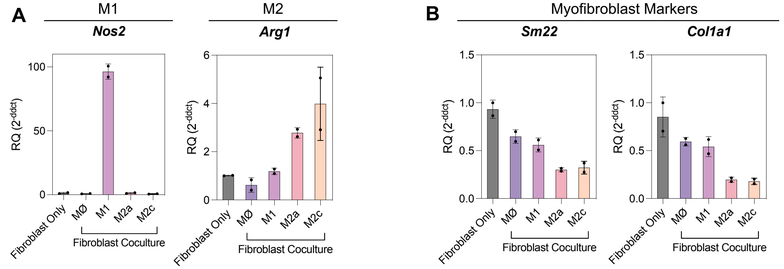

Figure 6: M2 AMs suppress myofibroblast expression. (A) Coculture with fibroblasts does not affect macrophage phenotype as noted by elevated expression of M1 marker *Nos2* and M2 marker *Arg1* in their respective polarized conditions. **(B)** Myofibroblast markers *Sm22* and *Col1a1* are downregulated when fibroblasts are cocultured with M2a and M2c macrophages.

FUTURE DIRECTIONS

- Study different pathways involved in the crosstalk between macrophage and fibroblast during lung injury using knockout models.
- Conduct an organoid assay with epithelial cells and refined macrophage population during injury to study the role of macrophage in the epithelial cell proliferation and repair

RERFERENCES

1. Davies, L. C., Jenkins, S. J., Allen, J. E., & Taylor, P. R. (2013). Tissue-resident macrophages. Nature immunology, 14(10), 986–995. https://doi.org/10.1038/ni.2705
2. Lendeckel, U., Venz, S., & Wolke, C. (2022). Macrophages: shapes and functions. Chemtexts, 8(2), 12. https://doi.org/10.1007/s40828-022-00163-4
3. Yao, Y., Xu, X. H., & Jin, L. (2019). Macrophage Polarization in Physiological and Pathological Pregnancy. Frontiers in immunology, 10, 792. https://doi.org/10.3389/fimmu.2019.00792
4. Fakoya, A. O., Naeem, A., & Pierre, L. (2022, October 10). Histology, alveolar macrophages. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK513313/
5. Zepp, J. A., & Morrisey, E. E. (2019). Cellular crosstalk in the development and regeneration of the respiratory system. Nature reviews. Molecular cell biology, 20(9), 551–566. https://doi.org/10.1038/s41580-019-0141-3
6. She, Y. X., Yu, Q. Y., & Tang, X. X. (2021). Role of interleukins in the pathogenesis of pulmonary fibrosis. Cell death discovery, 7(1), 52. https://doi.org/10.1038/s41420-021-00437-9

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