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Topic Category: 4033-ASIP EXTRACELLULAR MATRIX IN PATHOBIOLOGY

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Presentation Preference: Indifferent

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Sponsor's Society: Pathology - American Society for Investigative Pathology (ASIP) - Host Society
Keywords: 1. wound healing 2. cell crosstalk 3. chemokine
Awards: ASIP Trainee Travel Award

Bi-directional Macrophage-Fibroblast Crosstalk Directs Wound Resolution Factors

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Introduction: During the wound repair process, fibroblasts secrete abundant extracellular matrix (ECM) proteins and participate in remodeling of the provisional ECM. Macrophages and other immune cells also play an important role in all stages of wound healing. Classically activated macrophages (CAMs/M1) contribute to inflammation early on, while alternatively activated macrophages (AAMs/M2) secrete ECM-degrading enzymes including matrix metalloproteinases (MMPs) during the resolution phase. It is also understood that macrophages instruct fibroblast activities and that crosstalk is largely coordinated by secreted factors. However, the differential effect of CAMs and AAMs on fibroblasts remains elusive. CXCR3 is a chemokine receptor known to modulate both fibroblast and macrophage activity during wound healing and its activation acts as a critical stop signal to halt continuous ECM remodeling by fibroblasts. Yet, the importance of CXCR3 in macrophage-fibroblast crosstalk during tissue repair remains explored. *Therefore, the goal of this study was to investigate the interplay between macrophages and fibroblasts and elucidate the role of CXCR3 signaling in this interaction.*

Methods: We differentiated naïve bone marrow-derived macrophages (M0) into either CAMs/M1 using IFN- γ and LPS, or AAMs/M2 using IL-4. To study the effects of secreted and cell-contact dependent factors, we used both indirect and direct co-culture systems. The role of CXCR3 in macrophages and fibroblasts was examined using cells from wild-type (WT) and CXCR3^{-/-} knockout mice.

Results: Cytokine profiling revealed that fibroblasts treated with media conditioned (CM) by CAMs secreted higher levels of CXCR3 ligands CXCL10 and CXCL11 compared to untreated fibroblasts. Because both fibroblasts and macrophages express CXCR3, it was unclear which cell type might respond to these ligands. Investigation using qRT-PCR, showed that treatment with WT CAM CM resulted in increased fibroblast expression of MMP8 and MMP9 compared to no treatment. Fibroblast induction of MMPs by WT CAM CM persisted when CXCR3^{-/-} fibroblasts were treated with WT CAM CM, but was greatly diminished when fibroblasts were cultured with CXCR3^{-/-} CAM CM, suggesting that this crosstalk requires CXCR3 expression on CAMs but not on fibroblasts. The influence of macrophage subtypes on MMP8, MMP9, and TGF- β -induced α -SMA and ECM protein production was observed by IF staining.

Conclusions: Our data indicate that CAMs induce fibroblast expression of CXCR3 ligands and key matrix degrading enzymes MMP8 and MMP9, with MMP expression requiring CXCR3 expression on CAMs. Thus, our study suggests for the first time that paracrine interaction between fibroblasts and macrophages dictate the effector functions of fibroblasts and the wound healing process.

Support or Funding Information

Supported by grants from NIAMS and NIAD (AR68317 CCY and R56A1097679 NI), and support in kind from the Pittsburgh VA Medical Center (AW), the School of Nursing, and McGowan Institute of Regenerative Medicine (BNB, CCY).