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Characterizing and Engineering Mesenchymal Stromal Cells for Anti-Microbial Therapy

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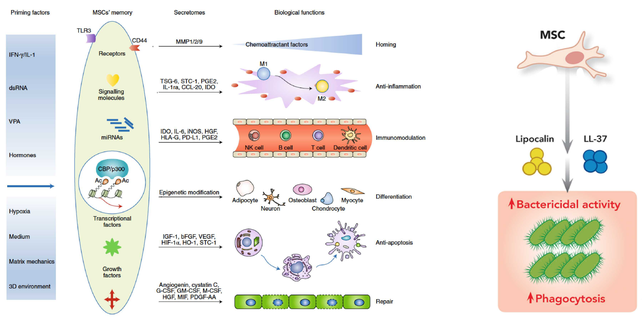
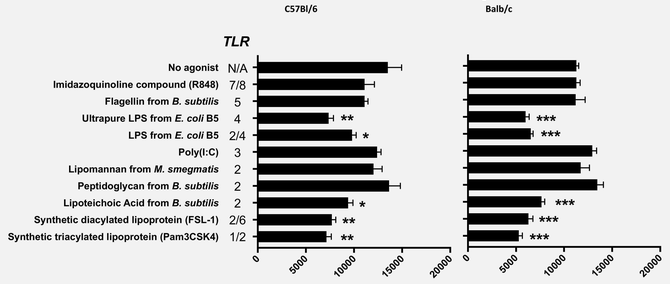
Abstract

Stem cell-based therapies are rapidly gaining traction in a number of areas of medicine, including in the response to the growing need for novel treatments for infectious disease. Mesenchymal stem cells (MSCs) are an extremely versatile adult stem cell type, having regenerative, anti-bacterial and immunomodulatory properties, all of which are vital for defending against infectious agents and remediating sites of infection. However, this versatility comes at a price – MSCs represent a heterogeneous spectrum of cell types, with lab-, strain- and context-specific differences in surface markers and properties. Our goal is to develop a system to regulate and deploy these various MSC properties without losing the inherent versatility. To achieve this, we are using transcriptomics and proteomics to characterize MSCs and related cell types from different sources. Using this integrated data to inform our experimental design, we use gene editing technology to both generate MSC-like anti-microbial cells from related cell types, and specifically activate target genes in MSCs to reliably enhance antibacterial activity. Showing that MSCs can be engineered while still maintaining their identity and versatility solidifies the status of MSCs as a major therapeutic not only for infectious disease, but for any medical conditions that require immunomodulation and/or regeneration.

MSCs have immunomodulatory and antibacterial properties



Stimulation of TLR2 and TLR4 primes MSCs and increases antibacterial activity



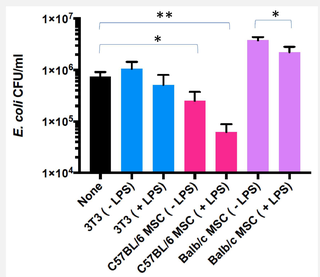
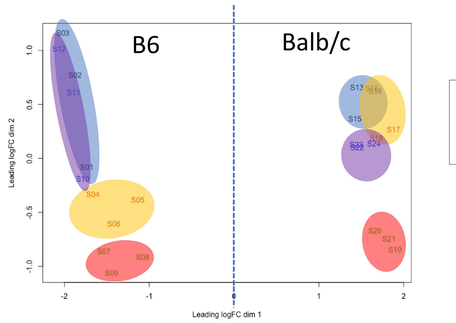
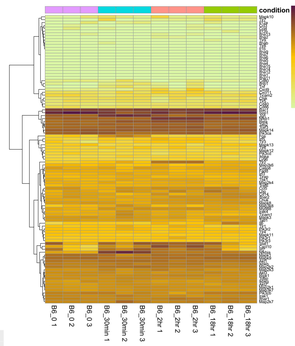


Fig 2. E. coli antibacterial assays after priming with TLR agonists and measured by Bactiter glo using MSCs from (**A**) C57BL/6 and (**B**) Balb/c. (**C**) CFUs from *E. coli* antibacterial assays of MSCs primed with LPS.

Transcriptional profiling of MSCs LPS stimulation reveals candidate genes regulating antibacterial effects





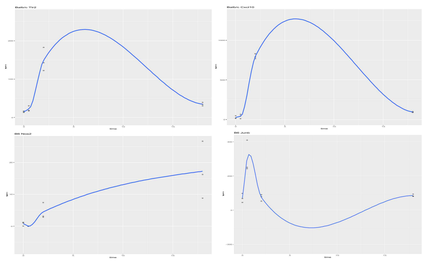
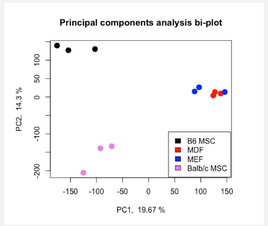
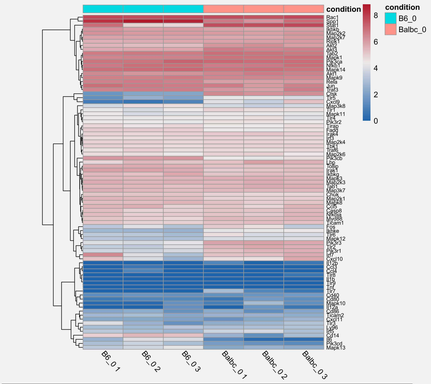
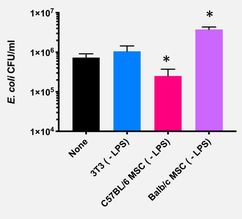


Figure 4. MSCs were stimulated with LPS and RNA seq was performed on cells collected at 0, 0.5 hr, 2 hr, and 18 hrs of exposure. (**A**) Transcriptional profiles of different time points clustered into groups. (**B**) Heat map depicting TLR signaling gene counts during LPS-time course. (**C**) Examples of gene patterns during time course.

MSCs from different mouse strains have different immunomodulatory and antibacterial properties







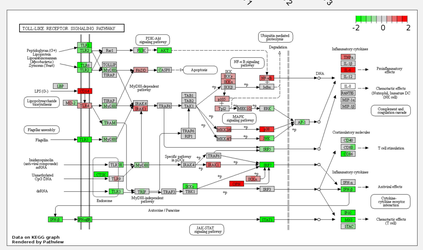
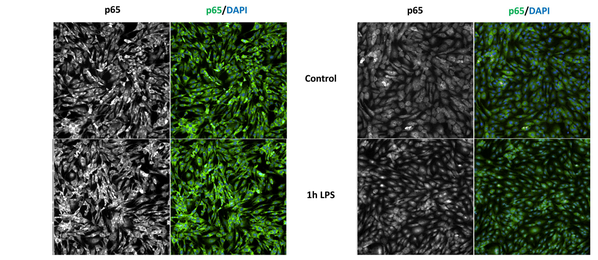
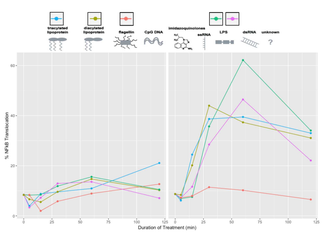
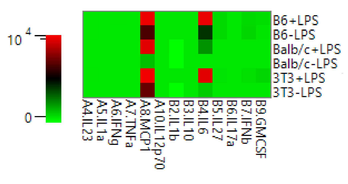


Fig 1. Bone marrow derived MSCs from Balb/c and C57BL/6 mice have distinct transcriptional profiles (**A**), differentially express genes involved with innate immunity (**B,D**), and have different antibacterial phenotypes (**C**).

TLR stimulation leads to differential NFkB translocation and cytokine production between mouse MSCs







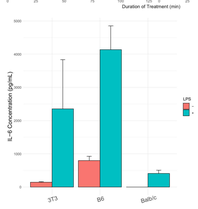
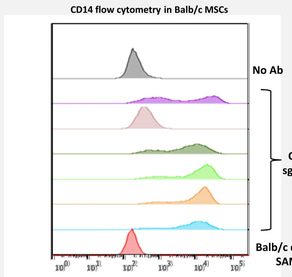
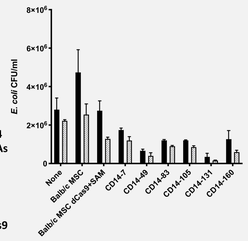
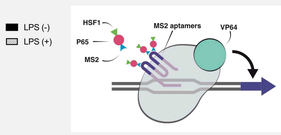


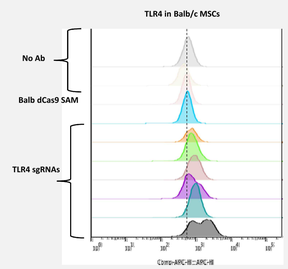
Figure 3. Stimulation of MSCs by TLR agonists leads to nuclear translocation of NFkB via p65 immunofluorescence in Balb/c MSCs but not C57BL6 (**A,B,C**). Cytokine profiling of LPS stimulated cells reveals increases in IL6 and MCP1 (**D,E**).

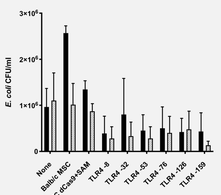
Engineering MSCs with CRISPR to be increase antibacterial activity











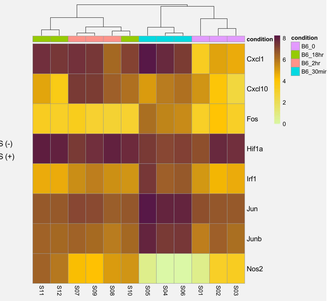


Figure 5. MSCs containing CRISPRa systems (SAM and VPR) are being utilized to increase antibacterial properties. MSCs were engineered to overexpress CD14 or TLR4 using CRISPR SAM system, and overexpression was checked with immunostaining and flow cytometry. Both overexpression lines promoted increased antibacterial behavior. Additional candidate genes to be tested from RNA seq analysis.

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References:

1. J. Q. Yin, J. Zhu and J. A. Ankrum, "Manufacturing of primed mesenchymal stromal cells for therapy." *Nature Biomedical Engineering* **3**, 90–104 (2019).
2. A. Monsel, Y.G. Zhu, S. Gennai, Q. Hao, J. Liu, J.W. Lee, "Cell-based therapy for acute organ injury: preclinical evidence and ongoing clinical trials using mesenchymal stem cells." *Anesthesiology* 121(5):1099-121 (2014).