Understanding the Effects of an Inflammatory Milieu on the Development of Mesenchymal Stem Cells: implication for clinical use
Lauren S. Sherman, Pranela Rameshwar
Rutgers University, RBHS, Newark, NJ: Dept. of Medicine, Hematology/Oncology, NJMS; School of Graduate Studies at NJMS

Abstract
Mesenchymal stem cells (MSCs), multipotent cells found in various adult tissues, are an attractive source of cells for cellular therapy and drug delivery, and for regenerative medicine. Reasons include their ease to expand, plasticity to generate cells of all germ layers, reduced ethical concerns, and ability to be available as ‘off the shelf’ cells for immediate use in transplantation. Further, these cells exert anti-inflammatory functions, home to areas of inflammation, and can be used to deliver drugs and small molecules in vivo. MSCs can respond differently to varying microenvironments to perform distinct immune functions. The microenvironment can also affect the developmental state of MSCs. Better understanding of how the microenvironment influences MSC multipotency is crucial for effective translational use of these cells in the clinic. This study tested the hypothesis that the changes in an inflammatory microenvironment will influence MSC function. To study these effects, MSCs were treated with either aspirin, a pan-anti-inflammatory mediator, or conditioned media from an in vitro model of graft versus host disease (GvHD). The GvHD model was generated based on a modified two-way mixed lymphocyte reaction. The cells were then assessed for phenotype, multi-lineage differentiation capacity, proliferation, and viability. The anti-inflammatory microenvironment resulted in increased senescence and a loss of the stem cell state. This in vitro analysis will help elucidate factors within the inflammatory milieu that alter MSC multipotency. Identifying these factors will allow for more controlled and effective clinical use of MSCs.

Background
• Primary tissue sources utilized for clinical use: bone marrow, adipose, umbilical cord
• Home to areas of inflammation
• Used in >1300 clinical trials
• Current “treatment to consider” for respiratory failure in COVID-19 patients (approved under FDA expanded access compassionate use)
• But what happens to the MSCs as inflammation subsides?

Aspirin Initiates Senescence in MSCs

Hypothesis
Changes in the inflammatory microenvironment will influence MSC function.

Methods
• Generate a 3D in vitro model of graft versus host disease (GvHD) based on a two-way mixed lymphocyte reaction (2-way MLR)
• Treat MSCs with aspirin, a pan-anti-inflammatory mediator, or media from the inflammatory GvHD model
• Assess MSC phenotype, multi-lineage differentiation capacity, proliferation, and viability

Figure 2: MSCs were stained with the cell tracker dye CMFDA for identification by flow cytometry and cultured in the 3D model in the absence (purple) or presence of peripheral blood mononuclear cells from one (pink, blue) or two (green) individuals.

Recovery of Cells From a 3D Model, with and without inflammation

Recovery of MSCs Form Apparent Colony Forming Unit – Fibroblasts

MSC Phenotype Differs with inflammation in a 3D Model

Figure 3: (A) When returned to traditional (2D) growth conditions, the MSCs from the 3D model form apparent colony forming unit – fibroblasts (CFU-F). Once disassociated, the MSCs return to normal growth patterns. (B) The cells maintain an MSC phenotype.

Decrease in MSC Viability in 3D Model

Summary
• MSCs survive within a 3D system, with and without an inflammatory micro-environment
• Culture in a 3D system appears to select for the more primitive MSCs, even in the absence of inflammation
• MSCs proliferate within the 3D culture system
• Inflammation supports MSC viability in the 3D culture system

Future Direction
• Determine the fate of MSCs as inflammation subsides, including changes in anti-inflammatory capacity
• Determine the effects of the inflammatory milieu over time
• Dissect the cellular and molecular causes of the observed effects on MSCs (role of specific cytokines, professional immune cells)
• Validate with MSCs derived from other tissues
• Emulate in vivo using models of traumatic injury

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