Understanding the Effects of an Inflammatory Milieu on the Development of Mesenchymal Stem Cells: implication for clinical use

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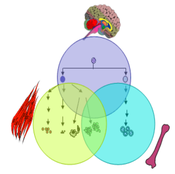


Abstract

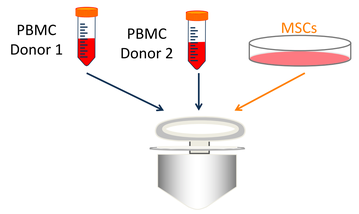
Mesenchymal stem cells (MSCs), multipotent cells found in various adult tissues, are an attractive source of cells for cellular therapy and drug delivery, and for regenerative medicine. Reasons include their ease to expand, plasticity to generate cells of all germ layers, reduced ethical concerns, and 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.

Hypothesis

Changes in the inflammatory microenvironment will influence MSC function.

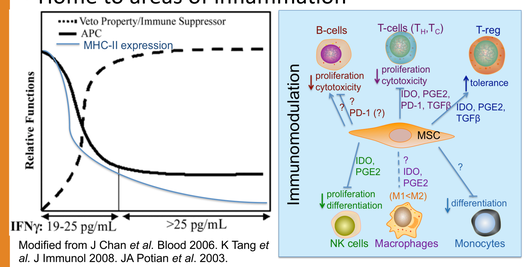
Methods

- 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



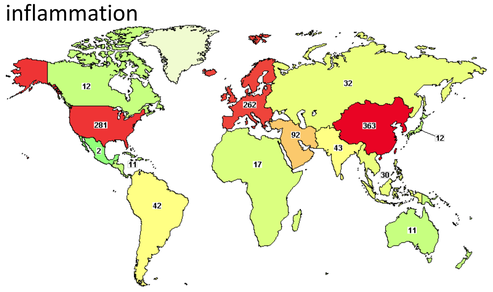
Background

- Primary tissue sources utilized for clinical use: bone marrow, adipose, umbilical cord
- Home to areas of inflammation



Modified from J Chan *et al.* Blood 2006. K Tang *et al.* J Immunol 2008. JA Potian *et al.* 2003.

- Act as anti-inflammatory cells in the presence of inflammation



clinicaltrials.gov

- 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

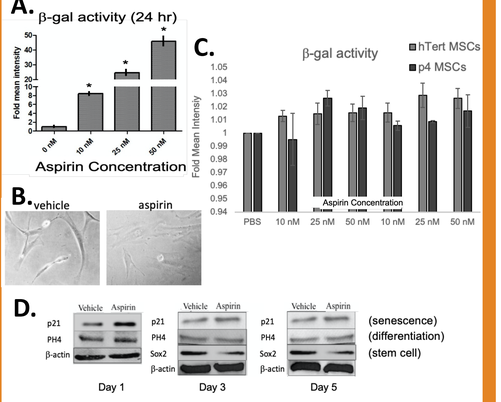


Figure 1: (A) MSCs were treated with varying concentrations of aspirin for 24 hours to determine the minimum concentration needed to initiate senescence. **(B)** Overnight treatment with 25 nM aspirin was sufficient to lose MSC morphology, **(C)** and subsequent days show loss of stem cell associated markers and increased markers of differentiation. **(D)** Increased senescence activity occurs within 2 hours of initial treatment in both bone marrow-derived MSCs (p4 MSCs) and immortalized adipose-derived MSCs (hTert MSCs).

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

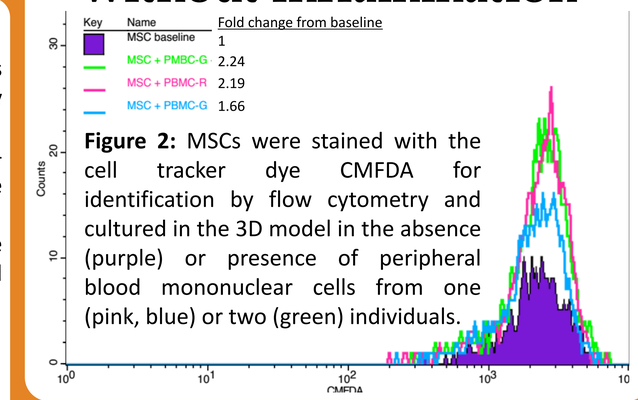


Figure 2: MSCs were stained with the cell tracker dye CMFDA for identification by flow cytometry and cultured in the 3D model in the absence (purple) or presence of peripheral blood mononuclear cells from one (pink, blue) or two (green) individuals.

MSC Phenotype Differs with inflammation in a 3D Model

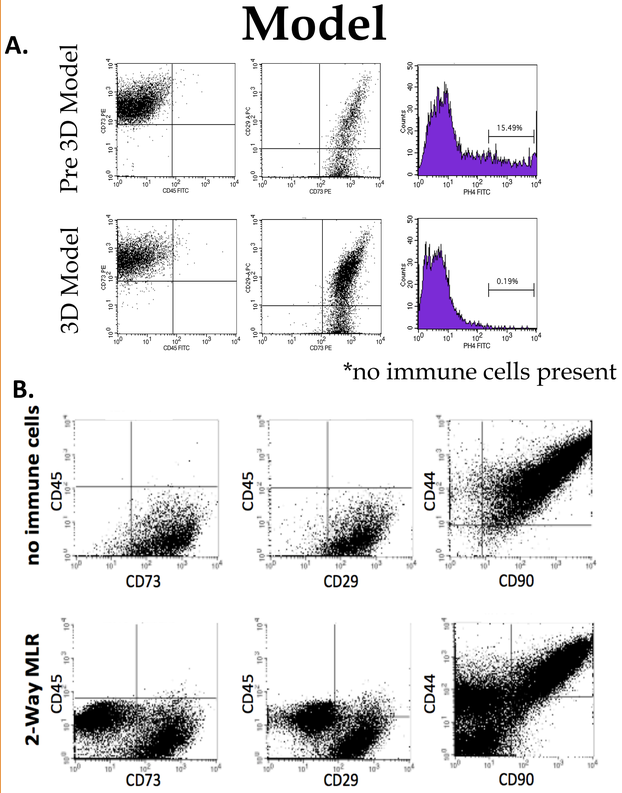


Figure 3: (A) Cells recovered from the 3D culture model were phenotypically MSCs as defined as $CD45^-$, $CD73^+$, $CD29^+$, $PH4^-$. **(B)** However, MSC phenotype differs between MSCs grown with or without the presence of inflammation in a 3D model.

Recovered MSCs Form Apparent Colony Forming Unit – Fibroblasts

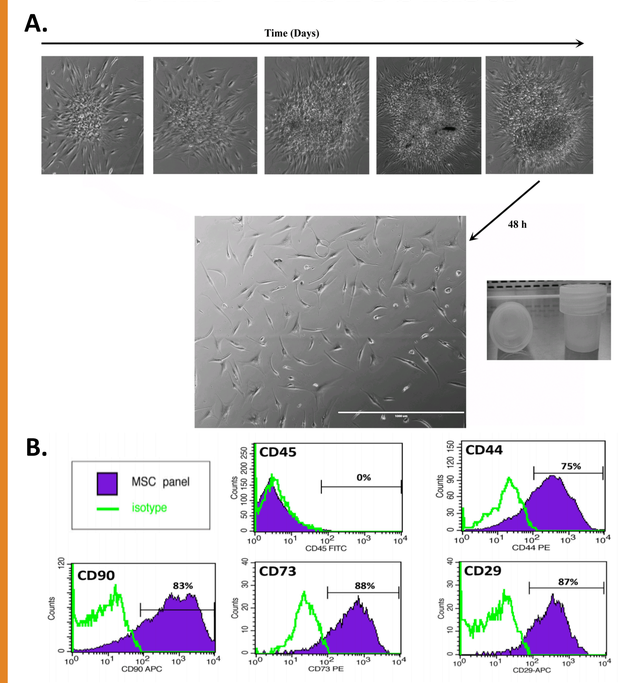


Figure 3: (A) When returned to traditional (2D) growth conditions, the MSCs from the 3D model form apparent colony forming unit – fibroblasts (CFU-F). Once disassociated, the MSCs return to normal growth patterns. **(B)** The cells maintain an MSC phenotype.

Decrease in MSC Viability in 3D Model

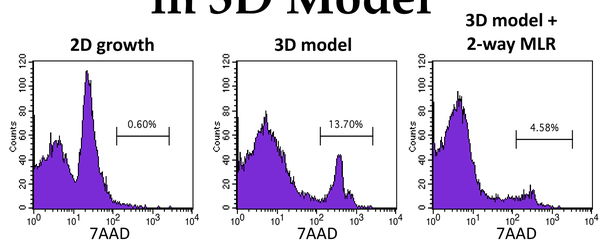


Figure 4: MSCs grown in a monolayer (2D) show a higher viability than those grown in the 3D model. This loss in viability may correlate with the PH4 positive (differentiated) population (Figure 3A). **(B)** The presence of an inflammatory milieu assisted in returning the 3D model culture's viability to that of the monolayer

Acknowledgements

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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