Mesenchymal Stromal Cell Conditioned Media Attenuates Macrophage Activation

Yaa Abu1, Suzette Riddle2, Karim, El Kasmi2, Kurt Stenmark2

1Princeton University, Princeton, New Jersey
2Pediatric Critical Care, Cardiovascular Pulmonary Laboratories, University of Colorado Anschutz Medical Campus, Aurora, Colorado

BACKGROUND

- Pulmonary Hypertension (PH) is a disease characterized by chronic inflammation and macrophage accumulation around remodeled vessels.

- In rodent models, macrophage depletion attenuates vascular remodeling.1

- Soluble factors produced by fibroblasts isolated from Pulmonary Hypertensive calves and humans (PH-Fibs) can activate macrophages to express pro-inflammatory mediators, including IL-1β.2

- Currently, treatment options for acute inflammatory lung diseases such as ARDS, and chronic diseases such as pulmonary hypertension are severely limited.3

- Mesenchymal Stromal Cells (MSC) possess anti-inflammatory properties that allow them to repair damaged tissues in certain models of acute and chronic lung injury.4,5,6

AIM

- Determine if pro-inflammatory activation of macrophages is attenuated by MSC Conditioned Media (MSC-CM).

HYPOTHESIS

- Treatment with MSC-CM will attenuate macrophage activation by stimulants associated with acute (LPS) and chronic (PH-Fib CM) lung inflammation via IL-10.

METHODS

- Bone Marrow Derived Macrophages (BMDM) Isolation and Differentiation:
  - Bone marrow collected from tibia and femur of mice. Grown in DMEM supplemented with 25% L-cell CM (sterile filtered), 10% FBS, L-glutamine, pyruvate, pen/strep, MEM NEAA, fungosone; maintained, fed for 5 days.

- Mesenchymal Stromal Cell Conditioned Media (MSC-CM) - provided by Advanced Cell Technologies, Marlborough, MA: Human embryonic stem cells (NIH approved); differentiated into mesenchymal stromal cells (MSC); grown 24 hours in 20% FBS-containing alpha MEM; collect media (MSC-CM);

- Stimulants:
  - LPS (200ng/mL);
  - PH-Fib-CM (24-hr incubation, serum-free);
  - IL-10 (10 ng/mL);

- RNA collection (Qiagen Mini Prep)

- Reverse transcriptase PCR (RT-PCR)

- Quantitative Real Time PCR (qPCR)

- Activating factor
  - LPS, PH-Fib CM

- Activating Pathway
  - NFkB

- MSC-CM, IL-10

- Activated Macrophage

- Inflammation/remodeling

RESULTS

- MSC-CM attenuates macrophage response to LPS

- IL-10 attenuates macrophage response to LPS

CONCLUSIONS/SPECULATIONS

- This study demonstrates that soluble factors within the MSC-CM attenuate macrophage activation, as it may occur in acute and chronic pulmonary inflammation.

- IL-10 is a candidate mediator within the MSC-CM.

- Future studies will be directed at investigating the mechanisms (detailed signaling pathways engaged) in order to develop suitable treatment and prevention strategies that interfere with inflammatory pathways in macrophages and other tissue resident cells such as fibroblasts.

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REFERENCES


