MSC THERAPIES FOR INFLAMMATORY LUNG DISORDERS

White Paper

15 Nov 2015

Next Gen MSC2 therapy evaluated in a murine LPS-induced acute lung injury model
# Table of Contents

**ABSTRACT** ............................................................................................................... 2  
  Background ........................................................................................................... 2  
  Methodology/Findings: ...................................................................................... 2  
  Conclusions:........................................................................................................... 2  

**INTRODUCTION** ................................................................................................. 3  

**STUDY DESIGN / METHODS** ........................................................................... 4  
  Mouse Model of ALI ......................................................................................... 4  
  Bronchoalveolar Lavage ............................................................................... 4  
  Lung Homogenization ..................................................................................... 5  
  Myeloperoxidase Activity Assay and Multiplex Immunoassay ................. 5  
  Study Description .......................................................................................... 6  

**RESULTS** ........................................................................................................... 6  
  MSC2 Therapy Improved Lung Integrity ....................................................... 6  
  MSC2 Therapy Attenuated Neutrophil Infiltration in the Lungs ............... 7  
  MSC2 Therapy Dampened Pro-inflammatory Mediators ......................... 8  

**SUMMARY** ....................................................................................................... 8  

**REFERENCES** ................................................................................................... 9
MSC Therapies for Inflammatory Lung Disorders

NEXT GEN MSC2 THERAPY EVALUATED IN A MURINE LPS-INDUCED ACUTE LUNG INJURY MODEL

ANTI-INFLAMMATORY MSC2 THERAPY SAFELY ATTENUATES INFLAMMATION, REDUCES NEUTROPHIL INFILTRATION, AND PREVENTS LUNG DAMAGE

ABSTRACT

Background: Mesenchymal stem cells [MSCs] have emerged as important regulators of inflammatory immune responses and represent attractive candidates for cell-based therapies for diseases that involve excessive inflammation. Acute lung injury [ALI] and acute respiratory distress syndrome [ARDS] are inflammatory conditions for which treatment is mainly supportive due to lack of effective therapies. Safety and promising effects of MSC therapies was demonstrated in early clinical trials of ARDS. However, standing in the way of implementing MSC-based therapies for any disease is the fact that current methods to prepare MSCs result in poorly defined mixtures of cells, yielding inconsistent products with variable therapeutic outcomes. We have developed a new technology [STaRT32™] that can consistently deliver uniform anti-inflammatory acting MSCs that we termed MSC2. In this study, the therapeutic effects of MSC-based therapy were assessed in a preclinical model of lipopolysaccharide [LPS]-induced ALI.

Methodology/Findings: In collaboration with Dr. Bruce Bunnell and Dr. Deborah Sullivan (Tulane University), we tested unprimed MSCs and our primed anti-inflammatory MSC2 in a murine model of ALI. MSCs were delivered to C57Bl/6 mice (0.75x10⁶ total cells/mouse) by oropharyngeal aspiration (OA) four hours after the animals were challenged with lipopolysaccharide (15 mg/kg). Mice were sacrificed 72 hours after LPS exposure, and lung histology examined for evaluation of inflammation and injury. Bronchoalveolar lavage fluid was analyzed to determine total and differential cell counts, total protein concentrations, and myeloperoxidase [MPO] activity. Inflammatory cytokine expression was measured for assessment of the degree of lung inflammation.

Conclusions: MSC2 cells shared a similar safety profile as naïve MSCs, with no adverse effects reported for 58 treated mice. Primed MSC2 administration attenuated inflammation and neutrophil infiltration in the lungs. There were decreased levels of leukocyte (e.g. neutrophil) migration into the alveoli and total protein concentration in the bronchoalveolar lavage fluid [BALF], and MPO activity after the induction of ALI.
following the MSC2 therapy. Additionally, the MSC2 therapy effectively suppressed the expression of pro-inflammatory cytokines and increased the anti-inflammatory cytokine IL-10. These findings along with our other completed preclinical studies demonstrating that MSC2 therapy leads to attenuated inflammation and alleviated pain, support advancing this technology for use in improved cell therapy of ALI/ARDS and other inflammatory conditions of the lung.

INTRODUCTION

Acute lung injury [ALI] is a common clinical occurrence that results from a number of localized and systemic pathological conditions including sepsis, trauma, shock, pneumonia, gastric aspiration, toxic ingestion, and pancreatitis1-6. ALI can progress into a life-threatening condition known as acute respiratory distress syndrome [ARDS] particularly in critically ill patients. ALI/ARDS is characterized by acute onset of overwhelming pulmonary inflammation, bilateral infiltrates, and diffuse alveolar damage. Inflammation may progress to the point of widespread pulmonary edema and poor lung compliance that ultimately result in severe hypoxemia and devastating respiratory failure1-6. Studies indicate that the age adjusted incidence of ALI/ARDS in the United States is 86.2 per 100,000 person-years, and the mortality rate is 36-44% due to lack of an effective therapy 1-6. Current treatment for ALI/ARDS is mainly limited to supportive care including mechanical ventilation with concomitant treatment of underlying diseases or initiating factors. Given the severe complications and high mortality rate of ALI/ARDS, a novel and more effective therapy is needed.

Mesenchymal stem cell [MSC]-based therapies have had success in clinical trials of diseases ranging widely from graft-versus-host to joint and cartilage disorders. MSCs naturally home to sites of injury, have no adverse events, and their healing effect is primarily mediated through instigating an anti-inflammatory environment specifically at the injured or inflamed sites.7 MSC-based therapies are also attractive new treatment candidates because MSCs from self (autologous) or universal (allogeneic) donor sources can be safely delivered since they do not express immune co-stimulatory molecules that elicit immune or graft rejection even in xenotransplantation.8 In our animal studies, human MSC-based therapy had long lasting therapeutic effects (several months) though the delivered human MSCs lingered only a few hours (<72h).9-21 However, standing in the way of implementing MSC-based therapies for any disease is the fact that current methods to prepare MSCs result in poorly defined mixtures of cells, yielding inconsistent products with variable therapeutic outcomes.

A means to overcome the problem of naïve MSC heterogeneity exists in the form of stimulating their Toll-Like Receptors [TLRs] to yield a homogeneous anti-tumor or anti-inflammatory cell population 22. In particular, via Stimulated Toll-like Receptor Technology [STaRT™], naïve MSCs are programmed to the anti-inflammatory MSC2 phenotype by selectively stimulating TLR3. MSC2 therapy has been successfully tested
in six (6) murine models of disease. In studies modeling painful diabetic peripheral neuropathy (pDPN), human MSC-based therapy dramatically alleviated pain and attenuated pro-inflammatory IL-6, TNFα and IL-1 while enhancing anti-inflammatory IL-10 levels.9-21

Because mixed naïve MSC-based therapies are safe and show promise in treating complex inflammatory diseases like ALI/ARDS and MSC2 provide more consistent therapeutic responses, it follows that MSC2-therapy offers an advantage over conventional naïve MSC in ALI/ARDS 1,22-28.

**STUDY DESIGN / METHODS**

**Mouse Model of ALI**

Eight- to ten-week-old female C57Bl/6 mice (National Cancer Institute-Frederick, Frederick, MD) were used for ALI induction via oropharyngeal aspiration (OA) delivery of LPS as previously described.28 Briefly, anesthetized mice (2% isoflurane, VetOne, Meridian, ID) were suspended by the cranial incisors, and LPS from *Escherichia coli* 055:B5 (15 mg/kg, Sigma-Aldrich, St. Louis, MO) was pipetted into the back of the throat (Figure 1). The tongue was extracted to full extension to prevent the swallowing reflex, and the nares were pinch Spain shut to force breathing through the mouth and subsequent LPS aspiration. Four hours after LPS exposure, human MSCs [hMSCs] (3.75 × 10⁵/75 L HBSS) were delivered similarly by OA, and 30 minutes later a second dose of an equal number of cells was administered for a total of 7.5 × 10⁵ cells. For the control, two doses of HBSS (75 µL each) were delivered. The animals were sacrificed 24 or 72 hours after ALI induction by anesthesia with 80 mg/kg ketamine plus 8 mg/kg xylazine followed by laparotomy and exsanguination via laceration of the inferior vena cava. The lungs were processed for histology, bronchoalveolar lavage collection, and RNA or protein isolation as described below. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee [IACUC] at Tulane University and conformed to the requirements of the Animal Welfare Act [AWA].

**Bronchoalveolar Lavage**

The lungs were cannulated with 20-gauge intravenous [IV] catheters (Exel International Medical Products, St. Petersburg, FL) immediately after exsanguination and gently washed five times with 530 µL (right lung) or 1 mL (whole lung) lavage buffer. The lavage buffer consisted of PBS supplemented with protease inhibitor cocktail (Roche, Indianapolis, IN) and 0.4 mM EDTA (Gibco), except that EDTA was not added when the lavage fluid was to be used in the in vitro mASC stimulation assay. The lavage fluid was spun at 1,500g for 5 minutes at 4°C to pellet the cells. Cells from all five lavage collections were pooled for total cell counting while the supernatant from the first lavage was (a) used to stimulate mASCs in vitro or (b) stored at −80°C for biochemical analysis.
The protein concentration in the bronchoalveolar lavage fluid (BALF) was measured using the micro bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL). For differential cell counts, the cells were spun onto glass slides by Cytospin (Thermo-Shandon, Wilmington, DE) and stained with a modified Wright-Giemsa stain (Diff-Quik, Fisher Scientific, Pittsburgh, PA). The numbers of neutrophils, macrophages, eosinophils, basophils, and lymphocytes were determined up to a total of 100 cells in three random fields per sample.

**Figure 1: Mouse Acute Lung Injury Model**

![Mouse Acute Lung Injury Model](image)

**Lung Homogenization**

Excised lung tissue was weighed and homogenized using a Bio-Plex cell lysis kit (Bio-Rad, Hercules, CA) as per the manufacturer’s instruction. The homogenates were centrifuged at 10,000g for 15 minutes, and the supernatant was aliquoted and stored at −80°C until analyzed. The protein concentration in the supernatant was measured by BCA assay.

Total RNA from cultured cells was isolated using RNeasy mini kit (Qiagen). Total RNA from lung was isolated from homogenized tissue in TriPure Isolation Reagent (Roche) and was purified with the RNeasy mini kit. The RNA was first treated with DNase I (Amplification grade, Invitrogen) and then converted into cDNA using iScript cDNA Synthesis Kit (Bio-Rad) following the manufacturer’s instructions in a PTC-200 Peltier Thermal Cycler (MJ Research, Ramsey, MN).

**Myeloperoxidase Activity Assay and Multiplex Immunoassay**

The BALF myeloperoxidase (MPO) activity assay was performed as previously described.28 Briefly, BALF was added to a reaction buffer containing 50 mM potassium phosphate (pH 6.0), 0.0005% (v/v) H₂O₂, and 0.167 mg/mL o-dianisidine dihydrochloride (Sigma-
Aldrich); the absorbance at 460 nm was monitored for 30 minutes using the Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT). Measurement of cytokines and chemokines in lung homogenates was performed by multiplex immunoassay using a Millipore mouse cytokine/chemokine 32-plex kit (Millipore, Billerica, MA).

Study Description

For this study, done in collaboration with Dr. Bruce Bunnell and Dr. Deborah Sullivan (Tulane University), we tested human naïve bone marrow MSC and primed MSC2 cells. At the time of disease induction, MSCs were delivered to C57Bl/6 mice (0.75x10^6 total cells/mouse) by OA four hours after the animals were challenged with lipopolysaccharide (15 mg/kg). Mice were sacrificed 72 hours after LPS exposure, and lung histology examined for evaluation of inflammation and injury.

**Table 1:** Preclinical safety & efficacy of MSC & MSC2 in ALI mice (72h study)

<table>
<thead>
<tr>
<th>Mouse ALI Model</th>
<th>MSC-based Therapy</th>
<th>Cell Dose</th>
<th>Frequency</th>
<th>Disease Impact</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS-induced BalbC and C57BL/6j n=80</td>
<td>Hu MSCs (n=18)</td>
<td>0.375x10^6 OA</td>
<td>2X 4h PDI</td>
<td>Mostly anti-inflammatory, reduced neutrophil infiltration</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Hu MSC2 (n=40)</td>
<td>0.375x10^6 OA</td>
<td>2X 4h PDI</td>
<td>Attenuated neutrophil infiltration into the lungs, reduced IL-6 and increased IL-10</td>
<td>None</td>
</tr>
</tbody>
</table>

PDI: Post disease induction

RESULTS

**MSC2 Therapy Improved Lung Integrity**

Levels of protein and total cell infiltrates in the bronchoalveolar lavage fluid (BALF) were used to assess the extent of vascular leakage after vehicle control naïve MSC or MSC2 treatment. Control groups with only HBBS (vehicle)-challenged mice were included to assess any changes in these levels due to the injection method or cell treatment. Values presented are relative to intact, untreated mice (Figure 2).
MSC2 Therapy Attenuated Neutrophil Infiltration in the Lungs

Administration of MSC2 significantly decreased the neutrophil infiltration as determined by cells stained with a modified Wright-Giemsa stain (Diff-Quick) and myeloperoxidase (MPO) activity in BALF when compared to vehicle control, 24 hours after LPS exposure (Figure 3).

**FIGURE 2**

**FIGURE 3**

**Untreated Control**

**Naive MSCs**

**MSC2**

**Total MPO activity, pmole/mL**
MSC2 Therapy Dampened Pro-inflammatory Mediators

Inflammatory mediators were measured 24 hours after PBS or LPS challenge from lung lysates by multiplex immunoassay (Figure 4). All protein levels were normalized to untreated mouse levels. MSC2 therapy enhanced anti-inflammatory IL-10 levels.

**FIGURE 4**

In all parameters tested the anti-inflammatory MSC2 therapy was more effective than the unprimed naïve MSCs while retaining the established safety profile of MSC therapies.

- MSC2 therapy reduced inflammatory cell infiltration (neutrophils)
- MSC2 therapy attenuated pro-inflammatory IL-6, TNFα, and IL-1β and increased anti-inflammatory IL-10
- MSC2 therapy improved lung integrity (less BALF protein and MPO)

Thus, the next generation MSC2 cell therapy safely attenuates inflammation and restores lung function offering a new, safe, cell-based therapy for ALI/ARDS.
REFERENCES


For more information, contact Commence Bio, Inc.

[www.commencebio.com](http://www.commencebio.com)

San Diego: (858) 373-8614

Baltimore: (443) 955-4262