Canadian Critical Care Forum

Cell and Gene Therapy are the Future

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Scientist, Institute of Medical Sciences and Collaborative Program in Genome Biology and Bioinformatics

November 1st 2014
Disclosures

CIHR MOP 106545
CIHR OCN 126573
CIHR MOP130331
MRI Early Researchers Award
PSI 0-35

Tissue Regeneration Therapeutics (TRT)
Paralysed man Darek Fidyka walks again after pioneering surgery
Medical team regrow cells of patient’s severed spine in breakthrough that offers hope to millions with disability

Walking again
Paralysed man Darek Fidyka can walk again after team used cells of patient’s olfactory bulbs and strips of nerve fibres to patch the damaged site

1. Cells from the patient’s olfactory bulbs in the brain were removed and grown in the lab.

2. The cells were injected into the spinal cord above and below the damaged site.

3. Strips of nerve fibres were taken from the patient’s ankle to form a bridge for the cells to grow across.
Stem Cell Research that is Shaping the Medical World

The stem-cell hope

Stem cells, which have the potential to develop into some or all of the specialized cells in tissues and organs, provide hope that they can replace damaged cells. There is a need to find new ways to harness them to treat various diseases.

After FDA Approval, Duchenne's Muscular Dystrophy Patient Receives First Umbilical Cord Stem Cell Treatment in the United States

Stem cells that release cancer-killing toxins offer new brain tumor treatment

Can Fat Cells Fix Thin Hair?

Fat cells may play a role in regulating hair growth

Stem cell therapy to treat diabetes

Stem Cell Therapy: Improving Quality of Life

UNDER THE LENS
THE FIELD
Stem cells, the drivers of regenerative medicine, are master cells that can replicate and grow into other cell types in the body. The therapy involves introducing them into damaged tissue for treating chronic diseases (type I diabetes, cardiac disorders) and incurable ones (Alzheimer's, Parkinson's disease). Stem cells can be derived from various sources like five-to six-day-old embryos, cord blood or adult tissues.

THE POTENTIAL
Worth $1.2 bln in 2012, it's growing at 30%. Expected to reach around $16 bln by 2017. The Indian market is estimated to be $600 mln by then. It's uncharted territory and as science advances, new segments will be created—from drug discovery to treatment. Nearly 4,300 clinical trials are ongoing in this field worldwide.

Stem cells offer hope to 26/11 victim, activist

NEURODEGENERATIVE DISEASE
Mesenchymal stem cells conditioned to secrete neurotrophic factors provide hope for Huntington disease.
Overview

• What are Stem Cells?
• Properties that favour mesenchymal stem/stromal cells use in Sepsis/ARDS
• Evidence from Pre-clinical models
• Some ‘caveats’
• Beyond stem cells
  • Genetically modified Stem Cells
• First Trials
• Potential and future directions
What is a Stem Cell?

A single cell that can replicate itself, or...

differentiate into many cell types.
Source of Mesenchymal Stem/Stromal Cells

- Bone marrow
- Brain
- Dental pulp
- Heart
- Placenta
- Synovium
- Skin
- Muscle
- Pancreas
- Kidney

POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

M Dominici¹, K Le Blanc², I Mueller³, I Slaper-Cortenbach⁴, FC Marini⁵, DS Krause⁶, RJ Deans⁷, A Keating⁸, DJ Prockop⁹ and EM Horwitz¹⁰

Table 1. Summary of criteria to identify MSC

<table>
<thead>
<tr>
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<th>Adherence to plastic in standard culture conditions</th>
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<tr>
<td>1</td>
<td>Positive (≥ 95% +)</td>
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<tr>
<td></td>
<td>Negative (≤ 2% +)</td>
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<td>CD105</td>
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<td>HLA-DR</td>
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<tr>
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<th>Phenotype</th>
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<tr>
<th>3</th>
<th>In vitro differentiation: osteoblasts, adipocytes, chondroblasts</th>
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<td></td>
<td>(demonstrated by staining of in vitro cell culture)</td>
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Revisiting the Definition of MSCs

- **Nomenclature**
- **What defines a (mesenchymal) stem cells**
  - Functional assay – capacity for multipotency
- **Stemness (multilineage differentiation potential)**
  - Progenitor vs. Stem
  - Stemness requirements for clinical use
- **Fresh vs. cultured MSCs**
  - Tissue vs culture “properties”
- **Markers**
  - Species Specific
  - Therapeutic Effect Specific
- **Heterogeneity**

A Keating, Cell Stem Cell, 2012
P Bianco, Nat Med, 2013
Rationale for Sepsis/ARDS

**Immuno Privileged Cells**

- Source of cells: Adipose tissue, Bone marrow, Placenta, Cord blood
- Age of cells: A) Young, B) Old
- Time of MSC administration: a) Pre-injury, b) At time of injury, c) Post-injury
- Effects on injury:
  - Reduced inflammation: IL-10, IL-4, IL-1α, IL-1β, IL-12, TNFα, IFNy
  - Structural recovery: Decreased permeability of endothelium and epithelium
  - Bacterial clearance: NO, TDO
  - Microenvironment changes: Secretion of different factors by EVs including microRNAs
  - Results are inconclusive:
    - 1h after endotoxin-alleviates injury [21]
    - 7 days after bleomycin-no effect on injury [5]
    - 60,120 days after irradiation-differentiated into myeloblasts [71]
- Aged MSCs do not perform as well as young MSCs:
  - Old cells fail to differentiate in vitro [59]
  - Young cells have more pluripotential markers, longer telomerizes and greater proliferative capacities [56]

i) Anti-inflammatory
ii) Not-immunosuppressive
iii) Anti-bacterial
iv) Reduce tissue injury
v) Promote repair

Huleihel et al., Expert Opin. Biol. Ther. (2013) 13(10)
The effect of MSC Source on Efficacy:

- Source of MSCs
- Site of Delivery
- Potency
- Quality
- Age
- Desired Therapeutic Effect
- Target of Impact
- Off-Site effects
- Recipient Factors (Endotypes)
- Dose (Number)
- Timing

Cell Biochem Funct 2013; 31: 271–280
Front. Immunol., 04 September 2013
<table>
<thead>
<tr>
<th>ALI models</th>
<th>Resource and route of application of MSCs</th>
<th>Improved outcome</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>IT LPS/rat</td>
<td>Intrapleural $1 \times 10^6$ rat BM-MSCs with challenge</td>
<td>Lung injury, histology and inflammation</td>
<td>[11]</td>
</tr>
<tr>
<td>IT LPS/mice</td>
<td>IP $1 \times 10^6$ human UC-MSCs 4 hr after challenge</td>
<td>Treg, survival time, body weight, histology and lung injury</td>
<td>[23]</td>
</tr>
<tr>
<td>OA LPS/mice</td>
<td>OA $2.5 \times 10^5$ human BM-MSCs 4, 4.5 hr after challenge respectively</td>
<td>Inflammation and cytokines</td>
<td>[24]</td>
</tr>
<tr>
<td>CLP/mice</td>
<td>IV $2.5 \times 10^5$ mouse BM-MSCs 6 hr after challenge</td>
<td>Mortality, injury, cytokine, and bacteria clearance</td>
<td>[25]</td>
</tr>
<tr>
<td>IT E. coli/mice</td>
<td>IT $1 \times 10^6$ human BM-MSCs 4 hr after challenge</td>
<td>Bacterial clearance and inflammation</td>
<td>[26]</td>
</tr>
<tr>
<td>CLP/mice</td>
<td>IV $1 \times 10^6$ mouse BM-MSCs 24 hrs before or 1 hr after challenge</td>
<td>Mortality and organ function</td>
<td>[27]</td>
</tr>
<tr>
<td>Endotoxin/</td>
<td>Instil $5 \times 10^6$ human MSCs 1 hr after challenge</td>
<td>Extravascular lung water, lung endothelial barrier permeability and alveolar fluid clearance</td>
<td>[28]</td>
</tr>
<tr>
<td>ex vivo perfused</td>
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<tr>
<td>human lung</td>
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<tr>
<td>IP LPS/mice</td>
<td>IV $5 \times 10^5$ mouse BM-MSCs 1 hr after challenge</td>
<td>Lung inflammation, injury and oedema</td>
<td>[29]</td>
</tr>
<tr>
<td>IT LPS/mice</td>
<td>IV $2.5 \times 10^5$ mouse BM-MSCs with or without overexpressing angiopoietin 1 30 min. after challenge</td>
<td>Inflammation, cytokine and permeability</td>
<td>[30]</td>
</tr>
<tr>
<td>IP LPS/mice</td>
<td>IT $7.5 \times 10^5$ mouse BM-MSC 4 hrs after challenge</td>
<td>Survival, and pulmonary oedema and permeability</td>
<td>[31]</td>
</tr>
<tr>
<td>IT endotoxin/mice</td>
<td>IV $2 \times 10^7$ mouse bone marrow mononuclear cells 1 hr after challenge</td>
<td>Lung inflammation, alveolar collapse and interstitial oedema</td>
<td>[32]</td>
</tr>
<tr>
<td>IT E. coli/mice</td>
<td>IT $1 \times 10^5$ human UC-MSCs 3 hr after challenge</td>
<td>Lung histology, inflammation and cytokine production</td>
<td>[33]</td>
</tr>
<tr>
<td>IP LPS/rat</td>
<td>IV $5 \times 10^5$ human UC-MSCs 1 hr after challenge</td>
<td>Survival rate and inflammation</td>
<td>[34]</td>
</tr>
<tr>
<td>IT E. coli/mice</td>
<td>IT $7.5 \times 10^5$ mouse BM-MSCs 4 hr after challenge</td>
<td>Survival and lung injury</td>
<td>[35]</td>
</tr>
<tr>
<td>IT LPS/mice</td>
<td>IV $3 \times 10^5$ human orbital fat-derived MSCs 20 min. after challenge</td>
<td>Inflammation and permeability</td>
<td>[36]</td>
</tr>
<tr>
<td>IN LPS/mice</td>
<td>IT $2 \times 10^5$ mouse BM-MSCs 4 hr after challenge</td>
<td>Alveolar leucocytosis, protein leak, surfactant secretion and mortality</td>
<td>[37]</td>
</tr>
</tbody>
</table>

IV: intravenous; IP: intra-peritoneal; IT: intra-tracheal; CLP: caecal ligation and puncture; OA: oropharyngeal aspiration; IN: intranasal; LPS: lipopolysaccharide; MSCs: mesenchymal stem cells; UC-MSCs: umbilical cord MSCs; BM-MSCs: bone marrow derived-MSCs; ALI: acute lung injury.
Aging Mesenchymal Stem Cells Fail to Protect Because of Impaired Migration and Antiinflammatory Response

Martha L. Bustos¹,²,³, Luai Huleihel¹,²,³, Maria G. Kapetanaki¹,²,³, Christian L. Lino-Cardenas¹,², Lyle Mroz¹,², Bryon M. Ellis¹, Bryan J. McVerry¹, Thomas J. Richards¹,², Naftali Kaminski¹,², Nayra Cerdenes¹,², Ana L. Mora¹, and Mauricio Rojas¹,²,⁴ Am J Respir Crit Care Med Vol 189, Iss 7, pp 787–798, Apr 1, 2014
Mesenchymal Stromal (Stem) Cell Therapy Fails to Improve Outcomes in Experimental Severe Influenza

Ilyse Darwish¹,², David Banner³, Samira Mubareka⁴, Hani Kim², Rickvinder Besla¹, David J. Kelvin³, Kevin C. Kain¹,²,³,⁵, W. Conrad Liles¹,²,³,⁵,⁶

MSCs from Dr. Darwin Prockop, Texas A&M

Figure 1. Neither prophylactic nor therapeutic administration of mMSCs affected weight loss or improved survival in two models of experimental severe influenza. 7–10 week-old male C57Bl/6 mice were (A,B) infected with 425 EID₅₀ influenza A/PR/8 virus and administered
Influenza causes prolonged disruption of the alveolar-capillary barrier in mice unresponsive to mesenchymal stem cell therapy

Jeffrey E. Gotts, Jason Abbott, and Michael A. Matthay
Mechanisms of mesenchymal stromal cell immunomodulation

Activation by inflammatory mediators determines the effector mechanisms utilized by MSCs.
Mesenchymal Stem Cells Reduce Inflammation while Enhancing Bacterial Clearance and Improving Survival in Sepsis

MSC administration to septic mice results in:

(i) decreased 7 day mortality
(ii) reduced levels of circulating and pulmonary pro-inflammatory mediators
(iii) decreased pulmonary vascular leak
(iv) increased bacterial clearance

Beneficial effects are associated with profound transcriptional changes in sepsis-target organs

Treatment rather than prophylaxis
Transcriptional reconstitution of mitochondrial and bioenergy-related pathways

A. GSEA Identified Mitochondrial Related Genes

B. Network Analysis Showing Mitochondrial Related Pathways Up-regulated after MSC treatment

dos Santos et al Am J Pathol 2012
Deletion of Parkinson’s Related Gene Protects Against Experimental Bacterial Sepsis
Deletion of Parkinson’s Related Gene Enhances Bacterial Clearance

Amatullah et al. manuscript submitted, 2014
Genetic Manipulation of MSCs to Enhance Anti-Bacterial Potential

Knockout Mice → Mesenchymal Stem Cells → Conditioned Medium

Mouse MSC Conditioned Medium
7 day survival curve

- Sham + control medium
- Sham + WT MSC cond. medium
- Sham + MSC cond. medium
- CLP + control medium
- CLP + WT MSC cond. medium
- CLP + MSC cond. medium

Fluid Resuscitated + Buprenorphine + Imipenem
MSCs as Vehicles of Gene Delivery

Specific Therapeutic Effects

Tomchuck et al. Fron Cell Infect, 2012
Prevention of LPS-Induced Acute Lung Injury in Mice by Mesenchymal Stem Cells Overexpressing Angiopoietin 1

Shirley H. J. Mei, Sarah D. McCarter, Yupu Deng, Colleen H. Parker, W. Conrad Liles, Duncan J. Stewart

Vascular stabilization by Ang1 - destabilization by Ang2

Endothelial cell
Matrix
Pericyte

Image from Wihuri Research Institute
The functional study of human umbilical cord mesenchymal stem cells harbouring angiotensin-converting enzyme 2 in rat acute lung ischemia-reperfusion injury model
The Toll-like Receptor 3 Ligand, Poly(I:C), Improves Immunosuppressive Function and Therapeutic Effect of Mesenchymal Stem Cells on Sepsis via Inhibiting MiR-143

XIAOYIN ZHAO, DAN LIU, WEI GONG, GUANGFENG ZHAO, LIU LIU, LIU YANG, YAYI HOU

A

CLP → C-MSCs/P-MSCs/P-143-MSCs → Hour 0 → Hour 1 → Hour 25 → Sacrifice

B

Survival (%)

- Sham
- CLP
- C-MSCs
- P-MSCs
- P-143-MSCs

Time (d)
Identification of Therapeutically Relevant miRNAs

Treatments:
- Sham
- CLP
- MSC

Expression Profiles from Limma

N = 72 samples

Illumina Mouse WG 6.0v2

RNA Processing & Biogenesis
Protein Processing and Biogenesis
Inflammation & Immunity
Cell Permeability and Mobility
Arginine and Proline Metabolism
Mitochondrion & Oxidative Phosphorylation
Fatty Acid Metabolism
Muscle contraction
Insulin Regulation & Diabetes Pathways
Phosphatidylinositol signaling

Limma adjusted p ≤0.05 CLP+Placebo vs. CLP+MSCs
Identification of Therapeutically Relevant miRNAs

MSC-derived miRNA inhibitor

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<tr>
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<th>SAL HPF</th>
<th>SAL NC</th>
<th>SAL INH</th>
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<tr>
<td>LPS</td>
<td>HPF</td>
<td>NC</td>
<td>INH</td>
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Gali et al, manuscript submitted
Mesenchymal stem cells as a novel vaccine platform

Suzanne L. Tomchuck1‡, Elizabeth B. Norton1*,‡, Robert F. Garry1, Bruce A. Bunnell2,3, Cindy A. Morris1, Lucy C. Freytag1 and John D. Clements1

1 Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA, USA
2 Tulane Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, LA, USA
3 Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA, USA

FIGURE 1 | Strategy of modified MSC vaccination and possible MSC functions during vaccination. (A) MSC isolated from the bone marrow of human donors can be expanded in culture and modified by transfection using antigen(s)-encoding plasmid to express and secrete soluble proteins, including both cancer and microbial antigens. Parenteral immunization of these modified MSC could then provide protective immunity. (B) These modified MSC may carry out several possible functions after vaccination. Primarily, it is expected that they serve as antigen delivery vehicles or even antigen depots following immunization. Based on the literature, it is clear that MSC can also take a more active role in induction of adaptive immunity, including cytokine secretion, like IL-6, and/or antigen presentation through phagocytosis and MHC-loading of antigen for presentation to lymphocytes expressing cognate T-cell/B-cell receptors. These immunostimulatory functions may also be involved in MSC-based vaccinations.
Chemical Approaches to Stem Cell Biology and Therapeutics

Wenlin Li,¹ Ke Li,² Wanguo Wei,³ and Sheng Ding²,*
¹Department of Cell Biology, Second Military Medical University, Shanghai 200433, China
²Gladstone Institute of Cardiovascular Disease, Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158, USA
³Stem Cell and Regenerative Medicine Center, Shanghai Advanced Research Institute, Chinese Academy of Science, Shanghai 201210, China
Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study

Guoping Zheng¹, Lanfang Huang¹, Haijiang Tong¹, Qiang Shu², Yaoqin Hu³, Menghua Ge¹, Keqin Deng¹, Liuya Zhang¹, Bin Zou¹, Baoli Cheng³ and Jianquo Xu¹*¹

Abstract

Background: Recent studies have demonstrated that mesenchymal stem cells (MSCs) modulate the immune response and reduce lung injury in animal models. Currently, no clinical studies of the effects of MSCs in acute respiratory distress syndrome (ARDS) exist. The objectives of this study were first to examine the possible adverse events after systemic administration of allogeneic adipose-derived MSCs in ARDS patients and second to determine potential efficacy of MSCs on ARDS.

Methods: Twelve adult patients meeting the Berlin definition of acute respiratory distress syndrome with a PaO2/FiO2 ratio of < 200 were randomized to receive allogeneic adipose-derived MSCs or placebo in a 1:1 fashion. Patients received one intravenous dose of 1 x 10⁶ cells/kg of body weight or saline. Possible side effects were monitored after treatment. Acute lung injury biomarkers, including IL-6, IL-8 and surfactant protein D (SP-D), were examined to determine the effects of MSCs on lung injury and inflammation.

Results: There were no infusion toxicities or serious adverse events related to MSCs administration and there were no significant differences in the overall number of adverse events between the two groups. Length of hospital stay, ventilator-free days and ICU-free days at day 28 after treatment were similar. There were no changes in biomarkers examined in the placebo group. In the MSCs group, serum SP-D levels at day 5 were significantly lower than those at day 0 (p = 0.027) while the changes in IL-8 levels were not significant. The IL-6 levels at day 5 showed a trend towards lower levels as compared with day 0, but this trend was not statistically significant (p = 0.06).

Conclusions: Administration of allogeneic adipose-derived MSCs appears to be safe and feasible in the treatment of ARDS. However, the clinical effect with the doses of MSCs used is weak, and further optimization of this strategy will probably be required to reach the goal of reduced alveolar epithelial injury in ARDS.

Trial registration: Clinical trials.gov, NCT01902082

Keywords: Mesenchymal stem cells, Adipose-derived, Acute respiratory distress syndrome, Biomarkers
Summary

• Therapeutic Potential of MSCs
• Therapeutic Potential of genetic manipulated MSCs
• MSCs as Vehicles for Gene Delivery
• MSCs as Agents of “Discovery”
• MSCs as novel Vaccine Platforms

STEM CELLS

Stem Cell Scientists Awarded Nobel Prize in Physiology and Medicine

In what researchers view as validation of the field, the Nobel committee on Monday recognized pioneering contributions to stem cell science by John Gurdon and Shinya Yamanaka

By Alice Park @aliceparkny  |  Oct. 08, 2012  |  5 Comments
Collaborators:
Dr. Shirley Mei
Dr. Jack Haitsma
Dr. Srinivas Murthy
Dr. Pingzhao Hu
Dr. Tak Mak
Dr. Conrad Liles
Dr. Duncan Stewart
Dr. Phil Marsden

Dr. Yuexin Shan
Hajera Amatullah
Dun Yuan Zhou
Patricia Gali
Louis Zhou