Stem Cells for ARDS: Promise and Pitfalls

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CANADA
Disclosures

• Orbsen Therapeutics, LTD [Galway, Ireland]

• Tissue Regeneration Therapeutics Inc [Toronto, Canada]
What are STEM CELLS?

Stem cell → Differentiation → Mature cells

Stem cell

Terminology

**Totipotent stem cell** that is capable of differentiation to 3 germ layers of the embryo as well as extraembryonic tissues.

**Pluripotent stem cell** that is capable of differentiation to 3 germ layers of the embryo is derived from the cells of the inner cell mass (ICM).

**Multipotent stem cell** that is capable of differentiation into only a closely related family of cells.

St. Michael's
Inspired Care. Inspiring Science.

Shokeir et al Int J Urol 2010
Sources of Stem Cells

- Types of Stem Cells
  - Embryonic
  - Adult
    - Induced Pluripotent

- ‘Adult’ Stem Cells
  - Amniotic Fluid Stem Cells
  - Hematopoietic stem cells (HSC)
  - Mesenchymal stem cells
  - Endothelial stem/progenitor cells

- Resident Stem Cells within Organs
  - Lung stem cells
Embryonic Stem Cells

- Oocyte
- Sperm
- Totipotent Morula
- Blastocyst
- Human Fetus
- Pluripotent Inner Mass Cells

Examples:
- Circulatory System
- Nervous System
- Immune System
- Unipotent
Induced Pluripotent Stem Cells

NEJM 2006; Nobel Prize 2012
Therapeutic potential of iPSCs

Hayes et al, Critical Care 2012
Evidence for Human Lung Stem Cells

Jan Kajstura, Ph.D., Marcello Rota, Ph.D., Sean R. Hall, Ph.D., Toru Hosoda, M.D., Ph.D., Domenico D’Amario, M.D., Fumihiro Sanada, M.D., Hangqiao Zheng, M.D., Barbara Ogórek, Ph.D., Carlos Rondon-Clavo, M.D., João Ferreira-Martins, M.D., Alex Matsuda, M.D., Christian Arraoto, M.D., Polina Goichberg, Ph.D., Giovanna Giordano, M.D., Kathleen J. Haley, M.D., Silvana Bardelli, Ph.D., Hussein Rayatzadeh, M.D., Xiaoli Liu, M.D., Ph.D., Federico Quaini, M.D., Ronglih Liao, Ph.D., Annarosa Leri, M.D., Mark A. Perrella, M.D., Joseph Loscalzo, M.D., Ph.D., and Piero Anversa, M.D.

ABSTRACT

BACKGROUND
Although progenitor cells have been described in distinct anatomical regions of the lung, description of resident stem cells has remained elusive.

METHODS
Surgical lung-tissue specimens were studied in situ to identify and characterize human lung stem cells. We defined their phenotype and functional properties in vitro and in vivo.

RESULTS
Human lungs contain undifferentiated human lung stem cells nested in niches in the distal airways. These cells are self-renewing, clonogenic, and multipotent in vitro. After injection into damaged mouse lung in vivo, human lung stem cells form human bronchioles, alveoli, and pulmonary vessels integrated structurally and functionally with the damaged organ. The formation of a chimeric lung was confirmed by detection of human transcripts for epithelial and vascular genes. In addition, the self-renewal and long-term proliferation of human lung stem cells was shown in serial-transplantation assays.

CONCLUSIONS
Human lungs contain identifiable stem cells. In animal models, these cells participate in tissue homeostasis and regeneration. They have the undemonstrated potential to promote tissue restoration in patients with lung disease. (Funded by the National Institutes of Health.)
Bone marrow stromal cells attenuate sepsis via prostaglandin E2–dependent reprogramming of host macrophages to increase their interleukin-10 production.

C57BL/6 Mice Randomised

CLP Sepsis

1 million Allogeneic BMSC’s

↓ Injury Severity

↑ Survival

Control Allogeneic cells

↑ Injury Severity

↓ Survival
Mesenchymal stem cells enhance survival and bacterial clearance in murine Escherichia coli pneumonia

Naveen Gupta,¹,* Anna Krasnodembskaya,²,* Maria Kapetanaki,¹ Majd Moulded,¹ Xinping Tan,¹ Vladimir Serikov,³ Michael A Matthay²

Gupta, Krasnodembskaya et al, Thorax 2012
Antibacterial action of MSCs

Gupta, Krasnodembskaya et al, Thorax 2012
Stem Cell Therapies – focusing on lung Repair?

Ware L, Matthay M, NEJM 2000

NUI Galway
OÉ Gaillimh
Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat

Gerard F Curley,¹,² Mairead Hayes,¹,² Bilal Ansari,¹,² Georgina Shaw,³ Aideen Ryan,³ Frank Barry,³ Timothy O’Brien,³ Daniel O’Toole,²,³ John G Laffey¹,²,³

Acute lung injury

ORIGINAL ARTICLE

Anaesthetise
Intubate

24hrs

Baseline Ventilation
20 min

VILI
35cmH₂O

Compliance ↓ed 50%

Recover and extubate

2 million MSCs

24hrs

24hrs

Anaesthetise

Surgery, Tracheostomy, Assessment of ALI

Curley et al, Thorax 2012
MSCs enhance Lung Repair

Curley et al, Thorax 2012
MSCs enhance injury resolution following VILI

Curley et al, Thorax 2012
Effects of Intratracheal Mesenchymal Stromal Cell Therapy during Recovery and Resolution after Ventilator-induced Lung Injury

A

Alveolar-arterial Oxygen Gradient (mmHg)

No Therapy  Vehicle  Fibroblast  IT MSC  IT CM  IV MSC

B

Respiratory Static Compliance (m/s/cmH2O)

No Therapy  Vehicle  Fibroblast  IT MSC  IT CM  IV MSC

C

Wet:Dry Ratio

No Therapy  Vehicle  Fibroblast  IT MSC  IT CM  IV MSC

D

Protein in BAL (ng/ml)

No Therapy  Vehicle  Fibroblast  IT MSC  IT CM  IV MSC
What about Human MSCs?
Experimental Plan

Anaesthetise
Intubate

Baseline Ventilation 20 min

VILI 35cmH₂O

4 million hMSCs

24hrs

Compliance ed 50%

Recover and extubate

Anaesthetise

Surgery, Tracheostomy, Assessment of ALI

Hayes, Masterson et al, unpublished Data
hMSCs enhance Lung Repair

Hayes, Masterson et al, Unpublished Data
hMSCs modulate Immune Response

Hayes, Masterson et al, Unpublished Data
Lowest effective hMSC dose

Hayes, Masterson et al, Unpublished
hMSCs reduce E. coli Lung Injury

Devaney et al, Unpublished Data
Don’t worry. I had ARDS too and MSCs cured me!

First clue that the latest medical breakthrough isn’t quite there yet.
Allogeneic human mesenchymal stem cells for treatment of *E. coli* endotoxin-induced acute lung injury in the ex vivo perfused human lung

Jae W. Lee, Xiaohui Fang, Naveen Gupta, Vladimir Serikov, and Michael A. Matthy

Department of Anesthesiology, Cardiovascular Research Institute, and Division of Pulmonary and Critical Care, University of California, San Francisco, CA 94143; and Children’s Hospital Oakland Research Institute, Oakland, CA 94609

Communicated by John A. Clements, University of California, San Francisco, CA, July 30, 2009 (received for review March 12, 2009)

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**Fig. 1.** Schematic diagram of the ex vivo perfused human lung and experimental protocol.

- Surgical preparation of one lung
- Begin perfusion without blood

- Measure AFC over 1 h in RUL or LUL (Control)
- Add 100 ml *Fresh Whole Blood* to Perfusate
- Lung Temp 36°C
- Apply CPAP

- Instill *allogeneic human MSC or MSC conditioned medium* to RML or LLL
- Measure AFC over 1 h in RML or LLL (endotoxin)
- Instill 0.1 mg/kg of *endotoxin* into the airspaces of the RML or LLL

CPAP 10 cm H$_2$O
95% O$_2$, 5% CO$_2$

Pulmonary Artery
Pressure = 10 - 12 mm Hg

Left Atrial
Pressure = 0 mm Hg

Perfusate Reservoir

37°C water bath

Peristaltic pump

Rate: 0.32 liters/min
Allogeneic human mesenchymal stem cells for treatment of *E. coli* endotoxin-induced acute lung injury in the ex vivo perfused human lung

Jae W. Lee¹, Xiaohui Fang², Naveen Gupta³, Vladimir Serikov⁴, and Michael A. Matthay⁵,⁶,⁷

¹Department of Anesthesiology, ²Cardiovascular Research Institute, and ³Division of Pulmonary and Critical Care, University of California, San Francisco, CA 94143; and ⁴Children’s Hospital Oakland Research Institute, Oakland, CA 94609

Communicated by John A. Clements, University of California, San Francisco, CA, July 30, 2009 (received for review March 12, 2009)

Control Lung Lobe

LPS Lung Lobe

LPS + MSC Lung Lobe

LPS + MSC CM Lung Lobe
How do MSCs work?
Bone marrow stromal cells attenuate sepsis via prostaglandin E$_2$–dependent reprogramming of host macrophages to increase their interleukin-10 production

Krisztián Németh$^{1,6}$, Asada Leelahavanichkul$^{2,6}$, Peter S T Yuen$^{2}$, Balázs Mayer$^{1}$, Alissa Parmelec$^{1}$, Kent Doi$^{2}$, Pamela G Robey$^{1}$, Kantima Leelahavanichkul$^{1}$, Beverly H Koller$^{4}$, Jared M Brown$^{5}$, Xuzhen Hu$^{2}$, Ivett Jelinek$^{3}$, Robert A Star$^{2,6}$ & Éva Mezey$^{1,6}$
Antibacterial Effect of Human Mesenchymal Stem Cells Is Mediated in Part from Secretion of the Antimicrobial Peptide LL-37

Anna Krasnodebskaya, Yuanlin Song, Xiaohui Fang, Naveen Gupta, Vladimir Serikov, Jae-Woo Lee, Michael A. Matthay

A

E. coli CFU (×10⁵/ml)

RPMI | MSC | NHLF

B

E. coli CFU (×10⁷/ml)

RPMI | MSC CM | NHLF CM

C

P. aeruginosa CFU (×10⁶/ml)

RPMI | MSC CM | NHLF CM

Unstimulated
E. coli stimulated
MSC anti-microbial effect LL-37 Dependent

A

E. coli CFU (x 10^7/ml)

RPMI  100 pg/ml  1 ng/ml  10 ng/ml  100 ng/ml

P. aeruginosa CFU (x 10^3)

RPMI  100 pg/ml  1 ng/ml  10 ng/ml  100 ng/ml

C

E. coli CFU (x 10^7/ml)

RPMI  + anti LL-37 Ab  + IgG

P. aeruginosa CFU (x 10^6/ml)

RPMI  + anti LL-37 Ab  + IgG

D

Krasnodembskaya et al, Stem Cells 2010
Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury

Mohammad Naimul Islam, Shonit R Das, Memet T Emin, Michelle Wei, Li Sun, Kristin Westphalen, David J Rowlands, Sadiqa K Quadri, Sunita Bhattacharya & Jahar Bhattacharya

Subpleural depth (μm): 6 6 10
Time after mBMSC instillation (h): 1 3 3

Islam et al, Nature 2012
Ready for Clinical Testing....?
Pitfalls…what is the key Mechanism?

- Role of Paracrine Mechanisms
  - Elements of MSC secretome
    - KGF
    - Anti-microbial Peptides
    - Prostaglandin E2 [? + Others]
  - Immune system ‘reprogramming’

- Role of Contact dependent versus independent mechanisms

- MSC RNA/Mitochondrial Transfer

- Transdifferentiation [very unlikely]

- Multiple competing Mechanisms of Action appear to exist
  - Is the MSC Mechanism of action dependent on Injury Type?
  - Are these mechanisms complementary or competing?
  - How important is the micro-environment to mechanism of action?
Fibrosis of open-lung biopsy (OLB) in patients with ARDS


<table>
<thead>
<tr>
<th>Result of OLB</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>16</td>
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<tr>
<td>Fibrosis and infection</td>
<td>29</td>
</tr>
<tr>
<td>Infection</td>
<td>28</td>
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<td>Diffuse alveolar damage</td>
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<td>Miscellaneous</td>
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<tr>
<td>Bronchioloalveolar carcinoma</td>
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<tr>
<td>Amiodarone toxicity</td>
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<tr>
<td>Intra-alveolar hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Allograft rejection</td>
<td>1</td>
</tr>
<tr>
<td>Drug toxicity</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatoid lung and mycobacterial infection</td>
<td>1</td>
</tr>
<tr>
<td>Acute eosinophilic pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>Carcinomatous lymphangitis</td>
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</tr>
<tr>
<td>Microangiitis</td>
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</tr>
</tbody>
</table>
MSCs can contribute to Lung Fibrosis

Yan X et al, Exp Hematol 2007
Other key ‘Translational Gaps’

- Should we give MSCs or just give the key Factors e.g. KGF?
- What is the optimal delivery route(s)?
  - Intravenous versus Intra-tracheal versus Other
  - Does this vary depending on e.g. etiology of Injury
- How well do MSCs work in more complex preclinical models?
- What is the optimal dosage regimen and therapeutic window?
- Address concerns in relation to potential downsides
  - Fibrotic potential?
  - MSC fate in humans?

Ware and Matthay, NEJM 2000
ARDS good ‘candidate’ disease for Cell Therapies
- Pre-clinical studies highly encouraging
- MSCs closest to clinical testing

Danger of attempting clinical translation in advance of better understanding of mechanisms of action

Need to learn much more about biology of ALI
- Enable harnessing of potential of these approaches

Ware and Matthay, NEJM 2000
LUNG-SAFE

Large observational study to UNderstand the Global impact of Severe Acute respiratory FailurE

A multicentre, prospective, observational, 4-week inception cohort study

Northern Hemisphere: Feb-Mar 2014
Southern Hemisphere: Jun-Aug 2014

Join us!
http://www.esicm.org/research/lung-safe
lung-safe@esicm.org