Conferred Susceptibility to VILI by Adoptive Bone Marrow Transfer of Myeloid Cells Lacking ATF3 Expression

Yuexin Shan, DunYuan Zhou, Ali Akram, Tatiana Maron-Gutierrez, Arthur S. Slutsky, David Hwang, Jack Haitsma, Claudia Dos Santos

Canadian Critical Care Forum
November 14th 2011
No Conflicts to Declare
Overview

- Background
  - ATF3, common promoter binding site in stretch-responsive genes
  - Susceptibility of ATF3 KO mice to VILI
  - Emerging role of ATF3 in Immunity

- Objective and hypothesis

- Adoptive bone marrow transfer

- Susceptibility of chimeras to VILI

- Summary
Ventilator Induced Lung Injury

Matrix Deposition
Matrix turn over

Levels of inflammatory mediators
Activation of coagulation cascade
Levels of growth factors

Fibrosis/fibrinolysis balance
Coagulum Resorption
Edema Resorption

Cell migration
Cell death/apoptosis
Cellular Proliferation
Cellular Infiltration

Injury and Altered repair and remodeling

dos Santos and Slutsky, Ann Rev Physiol 2006; and dos Santos ICM 2008
Effect of VILI on Survival from ARDS

• 861 patients
• Iatrogenic mortality due to VILI in the order of 9-10%
• Randomized to Low Vt ventilation vs. High Vt ventilation
• Reduction in Mortality from 40 to 31%

Cell-Stretch Model

Beas2b Cells
(immortalized distal bronchial airway epithelial cells)

- **Elongation 22%**
- **Cycles 30 cycles/min**
- **LPS (E.coli 10μg/ml)**
- **TNFα (10 ng/ml)**
- **Duration 4 hrs**

Identification of Stretch Sensitive genes

**Microarray analysis**

- **Stretch**
  - Stretch I
  - LPS + Stretch
  - TNFα + Stretch
- **Static Control**
  - LPS I
  - LPS + S I
  - TNF + S I

**RNA Isolation**

<table>
<thead>
<tr>
<th>Positive Regulation</th>
<th>Negative Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2rl2</td>
<td>Dusp1</td>
</tr>
<tr>
<td>Dnajb4</td>
<td>Schip1</td>
</tr>
<tr>
<td>Jun</td>
<td>Runx1</td>
</tr>
<tr>
<td>Znf238</td>
<td>Fn1</td>
</tr>
<tr>
<td>F112</td>
<td>C9orf125</td>
</tr>
<tr>
<td>Edf-R1</td>
<td>Dusp10</td>
</tr>
<tr>
<td>Epha2</td>
<td>EphA3</td>
</tr>
<tr>
<td>Dapk3</td>
<td>Tnfaip1</td>
</tr>
<tr>
<td>Akap12</td>
<td>Snf1Lk</td>
</tr>
<tr>
<td>IL6</td>
<td>Fign</td>
</tr>
<tr>
<td>Pthlh</td>
<td>Myc</td>
</tr>
<tr>
<td>Maff</td>
<td>Ppm2c</td>
</tr>
<tr>
<td>Traf4</td>
<td>Gabarap1</td>
</tr>
<tr>
<td>Cyld</td>
<td></td>
</tr>
</tbody>
</table>
Identification of Common Regulatory Element amongst cyclic stretch sensitive genes

<table>
<thead>
<tr>
<th>Gene Set Name</th>
<th>Reference</th>
<th>Size</th>
<th>NES</th>
<th>NOM p-val</th>
<th>FDR q-val</th>
<th>FWER p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCGCCTT,MIR-525,MIR-524</td>
<td></td>
<td>12</td>
<td>1.852</td>
<td>0</td>
<td>0.057</td>
<td>0.043</td>
</tr>
<tr>
<td>TGAYRTCA_V$ATF3_Q6</td>
<td>Donaldson and Gottgens, Nuc. Acid. Res. 2006</td>
<td>319</td>
<td>1.762</td>
<td>0</td>
<td>0.125</td>
<td>0.175</td>
</tr>
<tr>
<td>TGACGTCAV$ATF3_Q6</td>
<td>Wolfgang et al. Biol. Chem. 2000</td>
<td>155</td>
<td>1.735</td>
<td>0</td>
<td>0.124</td>
<td>0.247</td>
</tr>
<tr>
<td>GCAAGGA,MIR-502</td>
<td></td>
<td>52</td>
<td>1.717</td>
<td>0.0016393</td>
<td>0.128</td>
<td>0.325</td>
</tr>
<tr>
<td>GATAAGR_V$GATA_C</td>
<td></td>
<td>158</td>
<td>1.685</td>
<td>0</td>
<td>0.171</td>
<td>0.488</td>
</tr>
</tbody>
</table>

NES: Normalized Enrichment Score  
FDR: False Discovery Rate  
FWER p-val: Familywise error rate (FWER) p-value

Enrichment for genes with promoter regions [-2kb,2kb] around transcription start site containing conserved motifs for ATF3 binding sites

Activating Transcription Factor 3
ATF3 is regulated in vivo by VILI

a) Western Blot (Whole Lung)

b) Western Blot (Nuclear Extracts)

c) Immunohistochemistry

LV Ventilation + Saline

HV Ventilation + Saline

HV Ventilation + LPS

Akram et al. AJRCCM 2011
ATF3: Emerging roles in immunity

ATF3 inhibits NF-kappa B and AP1 transcription of pro-inflammatory genes

ATF3 KO confers susceptibility to VILI

- ATF3 KO mice are susceptible to VILI
  - Increased VILI-induced Lung Injury Scores
  - Increased VILI-induced Lung Edema
  - Increased VILI-induced Neutrophil infiltration
  - Increased VILI-induced pro-inflammatory mediator expression

Akram et al. AJRCCM 2011
Objectives and Hypothesis

**Rationale for research project:**
- ATF3 is expressed in both myeloid and non-myeloid cells
- Based on the gene deletion studies - ATF3 may protect against VILI

**Important Questions:**
- Which cells (myeloid vs. non-myeloid) induce protection from VILI?
- Can we transfer protection by bone marrow transplantation of ATF3 producing cells?

**Hypothesis:**
- As a major site of cytokine production, ATF3 expressing myeloid cells inhibited inflammation and alleviated lung injury.
Generation of ATF3 chimeras by adoptive bone marrow transfer

Donor
Male ATF3 deficient (ATF3 -/-)

Recipient
Female C57/b6 (ATF3 +/+)

Chimera: WT$^{ATF3}$
Parenchymal cells will be ATF3 +/+ 
Myeloid derived cells will be ATF3 -/-

Donor
Male C57/b6 (ATF3 +/+)

Recipient
Female ATF3 deficient (ATF3 -/-)

Chimera: ATF3$^{WT}$
Parenchymal cells will be ATF3 -/- 
Myeloid derived cells will be ATF3 +/+
Two-HIT VILI Protocol

- Chimeric mice – 20-25g
- **First Hit**: LPS inhalation 10µg/kg
- **Second Hit**: Mechanical Ventilation for 3 hrs: Vt = 6 ml/kg. PEEP =2 cmH₂O; FiO 0.4
All ATF3-Chimeras are susceptible to VILI

(b) Whole Lung Histology Two - hit (LPS inhalation plus HV ventilation)

(d) Lung Injury Score

Chimeras lacking ATF3 in either resident or circulating cells show increased lung injury
WT mice reconstituted with ATF3 KO bone marrow show increased neutrophil infiltration after VILI.

(a) Bronchoalveolar lavage Cytospin

(b) Total Neutrophil Count

Chimeras lacking ATF3 in myeloid cells show increased neutrophil infiltration.
ATF3 mice reconstituted with WT bone marrow show increased levels of inflammatory mediators.

Chimeras lacking ATF3 in parenchymal cells show increased levels of inflammatory mediators.
ATF3 KO mice reconstituted with WT bone marrow show increased pulmonary edema after VILI

Chimeras lacking ATF3 in resident cells show increased alveolar edema and decreased junctional protein expression.
Summary

Cell type (myeloid vs. non-myeloid) and susceptibility to VILI

- BOTH ATF3 chimeras are symptomatic
- Defects may be due to **cellular specific** function of ATF3
  - Lack of ATF3 expression in **myeloid** cells results in increased recruitment of neutrophils to the lung and alveolar spaces
  - Lack of ATF3 expression in **non-myeloid** cells results in increased expression of pro-inflammatory mediators, increased alveolar edema and decreased junctional protein expression

Bone marrow transplant and transfer of protection against VILI

- Not possible – repopulating the bone marrow with ATF3+/+ cells to mice lacking ATF3 does not correct the susceptibility to VILI

In conclusion, BOTH cell types uniquely contribute to protection against VILI
Acknowledgements

- dos Santos Lab
  - Yuexin Shan
  - Ali Akram
  - Tatiana Maron Gutierrez
  - Hussain Massoom
  - Suleiman Furmli

- Collaborators
  - Arthur S. Slutsky
  - Mingyao Liu
  - Jack Haitsma
  - Haibo Zhang