Lung Reperfusion Injury

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Guiding Principles in Medical Research

• Align your clinical and research activities

• Research must be clinically relevant

• Embrace unanticipated findings
Major Hurdles in Lung Transplantation

- Shortage of Donor Lungs
- Acute Graft Dysfunction
- Chronic Rejection
## Grading System

<table>
<thead>
<tr>
<th>Grade</th>
<th>P/F ratio</th>
<th>CXR infiltrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0:</td>
<td>&gt;300</td>
<td>absent</td>
</tr>
<tr>
<td>Grade 1:</td>
<td>&gt;300</td>
<td>present</td>
</tr>
<tr>
<td>Grade 2:</td>
<td>200-300</td>
<td>present</td>
</tr>
<tr>
<td>Grade 3:</td>
<td>&lt;200</td>
<td>present</td>
</tr>
</tbody>
</table>
Clinical Features

- Clinically known as **Primary Graft Dysfunction** or **Reperfusion Injury**
  - Early mortality - 30%
  - Increase MHC II expression and rate of rejection
  - Increases rates of airway complications and late graft failure - bronchiolitis obliterans
MOST CURRENT EFFORTS AND STRATEGIES TARGET PREVENTION
MAPK Activation

Transcription factor translocation

cytokine release

adhesion molecule expression

neutrophil recruitment

TISSUE INJURY
Remaining Fundamental Question:

How is oxidative stress transduced in the lung?

- Likely receptor mediated
- Must involve the alveolar macrophage
TOLL LIKE RECEPTORS

• Frontline in the innate immune system

• Toll-like receptor 4 (TLR-4) involved in other types of lung injury

• TLR-4 modulates reperfusion injury in other organs

• TLR-4 Well studied in macrophages
Strategies for inhibiting TLR-4 activation

- Pharmacologic inhibition
- Knockout mice
- Molecular deletion (siRNA)
Alveolar Macrophages Targeted with siRNA

Labeled siRNA

Controls
EFFECTS OF TLR-4 KNOCKDOWN

90% Reduction in Vascular Permeability

No difference from negative control

Permeability Index

NEGATIVE CONTROL

90 minutes ischemia
4 hours reperfusion
Research Must be Clinically Relevant….

Why don’t you investigate the impact of (bacterial products) on reperfusion injury?

Thomas Martin, MD
Lung Transplantation

Optimal Donor

- Age <55
- Clear chest x-ray
- \( \text{PaO}_2/\text{FiO}_2 > 300 \)
- <20 pk yr history
- Clear bronchoscopy
- Negative sputum gram stain

The Reality

- Lungs meet these criteria in only approx 12% of multiple organ donors
- 80% of donors have (+) bacterial cultures from bronchoscopy
TLR-4 activation

LPS

Oxidative Stress

IRAK4
IRAK
TRAF6

NFkB

MyD88

LUNG INJURY

Inflammatory Cell Infiltration
Proinflammatory Cytokine Production
Capillary Leak
VASCULAR PERMEABILITY INDEX

Positive Control (no LPS)

Negative Control (thoracotomy only)

LPS DOSE

Permeability

4000ng/kg

200ng/kg

15ng/kg

8ng/kg
LPS Pre-treatment reduces neutrophil infiltration

70% REDUCTION
Embrace unanticipated findings.....

Low dose LPS is protective against LIRI.
LPS Preconditioning

Possible Mechanism

Oxidative Stress

TRIF

TRAF3

IL-10

TBK

TRAM

Lung Protection

TIRAP

MyD88

IRAK4

IRAK

IRAK1

TRAF6

AP-1

NFkB

Proinflammatory Cytokine Production

Inflammatory Cell Infiltration

Capillary Leak

72%

60%

TLR-4

LPS

MyD88

MyD88

pp

IL-10

Lung Protection
LPS Preconditioning

Possible Mechanism

Oxidative Stress

TRIF

TRAF3

TRAM

IL-10

TBK

Lung Protection

TRAP

IRAK

IRAK1

IRAK4

TRAF6

AP-1

NFkB

MyD88

Proinflammatory Cytokine Production

Inflammatory Cell Infiltration

Capillary Leak

Inflammatory Stress

LPS
Clinical Relevance

• LPS pre-conditioning protects against lung reperfusion injury
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• Donor bacterial colonization n BAL is **NOT** necessarily a negative factor
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• LPS pre-conditioning protects against lung reperfusion injury

• Donor bacterial colonization n BAL is **NOT** necessarily a negative factor

• These phenomena are potentially relevant for all types of organ transplantation
Follow the lung transplant clinical and research programs on Twitter @SeattleLung and Facebook -- Seattle Lung Project