Immune approach to Primary Graft Dysfunction

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Disclosures

None
PRIMARY GRAFT DYSFUNCTION

- Incidence >50-70%
- Occurs within first 24 hours following transplant
- Characterized by respiratory failure
- Leading cause of short-term mortality
- Predominant risk factor for chronic rejection
PGD INDUCES CYTOKINE STORM AND ALLOIMMUNITY

Spectrum of PGD

Neutrophil-mediated allograft injury

Antibody-mediated rejection (hyperacute/acute)

Inadequate allograft preservation

Ischemia-reperfusion injury

Donor non-classical monocytes
Recipient classical monocytes

Donor Pneumonia
Donor Alveolar Macrophages

Donor-specific antibodies
Complement activation
Immune complex deposition
Monocyte/macrophage activation

Lung-restricted antibodies

Endothelial damage
Necroptosis
NEUTROPHILS MEDIATE PGD

PERFUSED HUMAN DONOR LUNGS CONTAIN MONOCYTES

Bharat et al, AJRCMB, Jan 2016

Zhikun et al, Science Transl Med, 2017
Demonstration of non-classical monocytes in the intravascular space of donor lungs

Human

Mouse

Pre-reperfusion

Post-reperfusion

NCM → CM → Neutrophils
NCM are visualized at sites of neutrophil recruitment and endothelial injury
LIPOSOMAL CLODRONATE DEPLETES Ly6C_{low} MONOCYTES IN PERFUSED LUNGS

Control

Clo-lip

Flushed Lung

Tissue monocytes

Patrolling endothelial-bound monocytes
Depletion of donor NCM abrogates neutrophil recruitment and ameliorates PGD.
Genetic depletion of donor NCM abrogates neutrophil recruitment

Donor Lung (Live CD45+ Ly6G− NK1.1− SiglecF− CD64− CD11b+)

WT

Nr4a1−/−

Nr4a1−/− + NCM

Donor Lung

Post-Transplant

% of CD45+ Cells in Donor Lung

Monocyte Composition in Donor Lung

Neutrophils in Allograft

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Nr4a1−/−</th>
<th>Nr4a1−/− + NCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+ Cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCM</td>
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</table>

* Indicates statistical significance.
Genetic deletion of fractalkine receptors on NCM inhibits their function.

Donor Lung (Live CD45+ Ly6G− NK1.1− SiglecF− CD64− CD11b+):

- Cx3cr1^{gfp/+}
- Cx3cr1^{gfp/gfp}

Donor Lung vs. Post-Transplant:

- % of CD45+ Cells in Donor Lung
- Monocyte Composition in Donor Lung
- Neutrophils in Allograft

Legend:
- Cx3cr1^{gfp/+}
- Cx3cr1^{gfp/gfp}

CM and NCM indicated by red and blue bars, respectively.
Unbiased transcriptomic profiling of NCM
Donor NCM produce MIP-2 in a MyD88-dependent fashion to recruit recipient neutrophils
Donor NCM produce MIP-2 following TLR2 stimulation

- TLR2 (Pam3CSK4)
- TLR3 (Poly I:C)
- TLR4 (LPS)
- TLR7/8 (R848)
- TLR9 (ODN1826)

MIP-2 (pg/ml)

No Stim control
- 0.1µg/ml
- 1µg/ml
- 10µg/ml
Monocyte subsets in mice and humans

**Classical Monocyte (CM)**

\[ \text{CCR2}^+ \text{Ly6C}^{\text{high}} \text{CX}_3\text{CR1}^{\text{low}} \]

**Nonclassical Monocyte (NCM)**

\[ \text{Ly6C}^{\text{low}} \text{CX}_3\text{CR1}^{\text{High}} \text{CCR2}^- \]
Depletion of recipient classical monocytes impairs neutrophil extravasation

Control

Depletion of host CM
Inflammatory host-derived classical monocytes are recruited from the spleen.
IL-1β production by host classical monocyte is necessary for neutrophil extravasation
IL-1β downregulates ZO-2 in endothelial cells disrupting endothelial barrier
Spleen not merely a monocyte reservoir – A new paradigm for splenic education of monocytes

A

Spleen CM
Bone Marrow CM

01 02 03 04 01 02 03 04

B

Fold increase
(Splenic CM/ Bone Marrow CM)
Bone marrow derived CM receive maturation signals from red pulp macrophages

Spleen harvest

% Total cells

Recipient Origin
Donor Origin

NCM CM T cells B cells RPM

Donor Origin

Spleen harvest

txp

12 um
Potential therapeutic targets

- Anti-IL1β therapy

Hsiao et al, J Clin Invest, 2018
NORTHWESTERN
62-yr female Emphysema 6L/min O₂
No traditional risks for PGD

CLEVELAND CLINIC
66-yr male, IPF, Pulmonary hypertension,
Prior LIMA graft, Cardiopulmonary Bypass

Ischemia time <3 hours for both

All Donor/ Recipient Cultures Negative

No DSA and Cross Match negative
<table>
<thead>
<tr>
<th></th>
<th>Pre-reperfusion</th>
<th>Post-reperfusion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10m</td>
</tr>
<tr>
<td>FiO₂(%)</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>O₂ sat(%)</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>PaO₂(mmHg)</td>
<td>80</td>
<td>150</td>
</tr>
</tbody>
</table>

Pre-transplant 1hr post-transplant
Autoantibody mediated rejection can mimic PGD

H & E STAINING

COMPLEMENT STAINING

Septal Neutrophils
Hyaline membrane
Alveolar Damage
**Lung-restricted antigens**

<table>
<thead>
<tr>
<th>Serum Autoantibodies</th>
<th>Pre-Transplant</th>
<th>Day of Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col V</td>
<td>Strong Positive</td>
<td>Strong Positive</td>
</tr>
<tr>
<td>K-α1 Tubulin (KAT)</td>
<td>Moderate Positive</td>
<td>Moderate Positive</td>
</tr>
<tr>
<td>Col I</td>
<td>Mild Positive</td>
<td>Mild Positive</td>
</tr>
<tr>
<td>Col IV</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
**TREATMENT**

**IVIG (1g/kg)**

**PLASMAPHERESIS**

**Eculizumab**

**Rituxamab (375mg/m^2)**

**Maintenance:** Tacrolimus, Mycophenolate, Prednisone

6-MONTH FEV1
71%
High incidence of pre-existing lung-specific autoantibodies in transplant recipients

Total study subjects: 142
Antibody positive: 41 (28.9%)
One: 4 (2.8%)
Two: 22 (15.5%)
Three: 15 (10.6%)

LRA predispose to PGD and chronic rejection

<table>
<thead>
<tr>
<th></th>
<th>PGD -ve</th>
<th>PGD +ve</th>
<th>Odds Ratio</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=142)</td>
<td>41(28.9%)</td>
<td>101(71.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody -ve</td>
<td>35 (34.5%)</td>
<td>66(65.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>6(19.4%)</td>
<td>35(80.6%)</td>
<td>3.09</td>
<td>1.2–8.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Two Positive</td>
<td>3(13.6%)</td>
<td>19(86.4%)</td>
<td>0.07</td>
<td>0.9–12.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Three Positive</td>
<td>1(6.7%)</td>
<td>14(93.3%)</td>
<td>7.4</td>
<td>0.9–58.9</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Lung autoantibodies induce rejection of murine lung grafts

Bharat et al, AJRCMB, Nov 2016
Severe PGD

Lung biopsy

- Antibody-mediated
  - LRA or DSA

- Monocyte/Macrophage mediated
  - Or DAD

Complement staining

Supportive therapy
- ECMO
- Novel targets
  - Anti-IL1β
  - Bisphosphonates

Complement inhibition

Antibody-directed therapy
- Plasmapheresis
- IVIG
- Rituximab
- Bortezomib
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