Transplant Immunity and Tolerance: Opportunities for Personalization?

Center for Applied Genomics and Precision Medicine Weekly Forum (3/14/2019)

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Outline

Transplant Immunity and Tolerance

Tolerance Stability

Individualizing Transplant Care
Life-long immunosuppression

**Metabolic side effects**
- Post transplant diabetes;
- Hyperlipidemia;
- Neurotoxicity;
- GI side effects;
- Decrease fertility

**Opportunistic infections**
- Viral;
- Fungal;
- Bacterial;

**Secondary malignancies**
- PTLD;
- Skin cancers;
- Tumors of solid organs
Transplant tolerance induction strategies: hematopoietic chimerism... alternatives?

- Requires recipient bone marrow conditioning;
- Long-term risk graft-versus-host disease remains present and formidable
Donor negative vaccination

Donor cells rendered apoptotic by chemical cross-linking using ethylene carbodiimide (ECDI-SP)

Approach

Spleens removed from donor mice

ECDI-fixed donor splenocytes injected i.v.

RBCs lysed

Single Cell Suspension

1 hour, 4°C

ECDI

ECDI-fixed Allogeneic Donor Splenocytes

?Graft Protection?
This approach effectively induces donor-specific tolerance.

ECDI-treated splenocytes (1x10^8), i.v.

Allogeneic transplant

ECDI-treated splenocytes (1x10^8), i.v.

Control (N=8)
- ECDI-treated BALB/c cells (N=10)
- Untreated BALB/c cells (N=5)
- ECDI-treated SJL cells (N=4)

Evidence of ECDI-SP undergo rapid apoptosis *in vitro*

In culture

Wang et al, Cell Transplant. 2015
Minardi S, unpublished
Evidence of ECDI-SP undergo rapid apoptosis \textit{in vivo}

Efferocytic receptors TAM (Tyro3/Axl/MerTK)

Axis #1: ECDI-SP $\rightarrow$ MFs $\rightarrow$ MDSCs $\rightarrow$ Tregs

Zhang et al, Am J Transplant. 2019
What about dendritic cells?

PKH-67 Dapi

10x

Spleen

Red pulp

MZ

White pulp

Red pulp МΦ

Erythrocyte

MZ МΦ

Microbe

Lymphocyte

Sinus-lining cells

Metallophilic МΦ

Sinus

Virus

Tingible body МΦ in GC

White pulp

FDC

Apoptotic debris
DCs mediate the tolerogenic effects of allogeneic ECDI-SPs

ECDI-SP  Dapi  CD11c

CD11+ DC depletion in CD11c-DTR mice

**Axis #2: ECDI-SP → DCs → T cell contraction**

**Ligands (ProS, Gas6)**

- **Mer**
- **ECDI-SP**
- **M**
- **F**

**Indirect T cells**

- **PD-L2, PD-L1**

**Expansion**

- **IL-10, CCL4**

**Depletion**

- **IFN-α, IFN-γ, ↑M-CSF**

**TOLERANCE**

Axis #3: ECDI-SP $\rightarrow$ T cell anergy

- **ECDI-SP** ligands (ProS, Gas6)
- **Mer** on MDSCs, Tregs
- **IFN-α, IFN-γ, M-CSF**
- **IL-10, CCL4**
- **Expansion** of Tregs
- **IDO, iNOS**

**Indirect T cells**
- **PD-L2, PD-L1**
- **Signal 1**
- **Signal 1 w/o signal 2**

**Direct T cells**
- **Depletion**
- **Anergy**

**Tolerance**

Proven efficacy in several small and large animal models

- **Murine allogeneic heart transplant**
  - Chen et al, AJT 2012
  - Bryant et al, JI 2014

- **Murine allogeneic kidney transplant**
  - Dangi et al, manuscript in preparation

- **Murine xenogeneic islet transplant**
  - Wang et al, Diabetes, 2012
  - Kang et al, Transplantation, 2017

- **Nonhuman primate allogeneic islet transplant**
  - Lei et al, AJT 2015
  - Singh et al, manuscript in revision

- **Humanized mice xenogeneic islet transplant**
  - Tangherlini-Lee et al, manuscript in preparation
Phase I/IIa “ETIMS (Establishing tolerance in MS)” trial

**Early R-R MS Patient**
(Pre-existing T cell response To 1-2 myelin epitopes)

Leukocytaphaeresis

- i.v. re-infusion of autologous Ag-PBLs (1x10^9)

- Antigen-coupled PBLs (Ag-PBL)

Cocktail of Immunodominant Myelin Peptides

ECDI

MBP_{13-32}  MBP_{83-99}  MBP_{111-129}  MBP_{146-170}  MOG_{1-20}  MOG_{35-55}  PLP_{139-154}

Lutterotti et al, Sci Transl Med 2013
Antigen-specific immune responses in ETIMS trial

10^3 Ag-PBL

10^5 Ag-PBL

10^7 Ag-PBL

10^8 Ag-PBL

5x10^8 Ag-PBL

10^9 Ag-PBL

Lutterotti et al, Sci Transl Med 2013
CMV infection disrupts MDSC differentiation and expansion

Dangi et al, Blood Adv. 2018
Systemic viral infections associated with acute rejections in humans

- **Kidney & liver recipients**
- **PBMCs, plasma**

![Chart showing single infections and coinfections detected in transplanted patients]

**Table 5. Relative risk of organ rejection.**

<table>
<thead>
<tr>
<th>Infection/Coinfection</th>
<th>Kidney Relative Risk</th>
<th>Liver Relative Risk</th>
<th>p Value</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>EBV</td>
<td>2.74</td>
<td>2.68</td>
<td>0.20</td>
<td>0.41</td>
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<tr>
<td>HCMV</td>
<td>4.28</td>
<td>40.33</td>
<td>0.06</td>
<td><strong>0.0013</strong></td>
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<tr>
<td>HHV6</td>
<td>5.60</td>
<td>6</td>
<td><strong>0.03</strong></td>
<td>0.20</td>
</tr>
<tr>
<td>HHV7</td>
<td>3.04</td>
<td>1.27</td>
<td>0.12</td>
<td>0.87</td>
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<tr>
<td>EBV/HCMV</td>
<td>4.86</td>
<td>NOT DETECTED</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>EBV/HHV6</td>
<td>4.86</td>
<td>NOT DETECTED</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td><strong>EBV/HHV7</strong></td>
<td><strong>5.60</strong></td>
<td>4.46</td>
<td><strong>0.03</strong></td>
<td>0.29</td>
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<tr>
<td>HCMV/HHV6</td>
<td>7.32</td>
<td>NOT DETECTED</td>
<td>0.12</td>
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<td>NOT DETECTED</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Relative risks with significant p are in bold.
Local (lung allograft) commensal virome

- Lung recipients
- BAL

Subject ID

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Bronchoalveolar lavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-03</td>
<td>11-09</td>
</tr>
<tr>
<td>11-15</td>
<td>11-21</td>
</tr>
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<td>11-27</td>
<td>12-02</td>
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<td>12-12</td>
<td>13-17</td>
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<td>13-19</td>
<td>13-20</td>
</tr>
<tr>
<td>13-28</td>
<td>13-31</td>
</tr>
</tbody>
</table>

Anelloviridae
Circoviridae
Herpesviridae
Parvoviridae
Polyomaviridae
Inoviridae
Myoviridae
Podoviridae
Siphoviridae

Days Post Transplantation

Log10 Reads

Eukaryotic viruses
Bacteriophages

Abbas et al, Am J Transplant 2018
Local (small bowel) commensal virome and GVHD in HSCT

- HSCT
- Stool

Legoff et al, Nat Med 2017
Intestinal commensal bacteria alter rejection kinetics of a distant transplanted organ

McIntosh et al, Microbiome 2018
Intestinal commensal bacteria alter rejection kinetics of a distant transplanted organ

Lee et al, Transplantation 2014
Systemic and organ-specific sites for microbiome identification

- Effects on graft rejection
- Effects on long-term graft outcome
- Effects on ischemic reperfusion injuries
  - Delayed graft function
  - Slow graft function
- Effects on infectious complications
- Effects by immunosuppression
Go straight to the tissue – what can we “see”?

Transplant kidney biopsy → Bulk RNA extraction → Microarray assessment → Normalization by reference set → Analyzed using predefined algorithms → “Molecular Scope Report”

Halloran et al, Am J Transplant 2017
## Prepare single cell suspension from kidney biopsies

<table>
<thead>
<tr>
<th>Biopsy No.</th>
<th>Biopsy A14</th>
<th>Biopsy A39</th>
<th>Biopsy A43</th>
<th>Biopsy A45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>1.5</td>
<td>1.2</td>
<td>1.0</td>
<td>1.3</td>
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<tr>
<td>Enzyme</td>
<td>0.25% Trypsin</td>
<td>0.25% Trypsin</td>
<td>0.25% Trypsin</td>
<td>0.25% Trypsin</td>
</tr>
<tr>
<td>Time</td>
<td>30 min</td>
<td>30 min</td>
<td>30 min</td>
<td>30 min</td>
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<tr>
<td>Total cells</td>
<td>75,000</td>
<td>46,000</td>
<td>44,000</td>
<td>64,000</td>
</tr>
<tr>
<td>Viability</td>
<td>91%</td>
<td>88%</td>
<td>96%</td>
<td>93%</td>
</tr>
</tbody>
</table>

Wu et al, JASN 2018
16 cell populations from an AMR kidney biopsy

A case of AMR from medical non-compliance

Wu et al, JASN 2018
Graft infiltrating cells in AMR vs. circulating cells

Wu et al, JASN 2018
Spatial relationships

Ligand-receptor relationship

Wu et al, JASN 2018
Beyond conventional assessment...

**Conventional assessment:**
- Demographics
- Panel reactive antibodies
- Donor specific antibodies

**Molecular matching:**
- Epitope matching
- Recipient SNP
- Donor SNP

**Semi-validated biomarkers:**
- PBMC cellular response
- PBMC immune phenotyping
- PBMC transcriptional signature
- Urinary chemokine

**Microbiome:**
- Intestinal
- Blood
- Urine

**Biopsy:**
- Single cell transcriptome
- Whole slide digital pathology

**Pharmacogenetics**

**Biorepository**

**Clinical outcome:**
- Early graft function
- Graft rejection
- Graft fibrosis
- Graft infection

**Neural network**
Deep Learning

**Predicative Model**

**Personalized transplant care**
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