Immunosuppression Withdrawal after Liver Transplantation: Hit or Miss???

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Organ Transplantation and Immunosuppression: A Love – Hate Relationship

- Indefinite, lifelong immunosuppression has been considered essential to prevent acute and/or chronic rejection of an allograft
- However, immunosuppression results in toxicities that result in long-term morbidity and mortality

Tolerance: The Holy Grail of Transplantation

- Tolerance is a state whereby an allograft remains normal in the absence of immunosuppression.
- The liver transplant setting may present a unique opportunity to study tolerance.

"Tolerogenic Effects of Porcine Liver Allografts"

RY Calne, RA Sells, JR Pena, DR Davis, PR Millard, BM Herbertson, RM Binns, DR Davis.

Pigs given no immunosuppression survive long periods with liver allografts; rejection has been minimal whether the donor and recipient were closely related or of different breeds. In contrast, rejection of both skin and kidney allografts by the pig is usually complete within 2 weeks.

The Liver: A Tolerogenic Organ

Basic Considerations
- Constant exposure to environmental antigens from the intestinal tract
- Hepatic microanatomy – distinctive local immune environment
- Abundant innate lymphocytes
  - NK / NK T cells
- Chronic infections
  - Hepatitis
  - Malaria
- Minimal to no impact of antibody-mediated rejection processes
- Recipient sensitization not assessed
- Crossmatching not performed
- Hyper-acute allo-immune responses rare
- Acute rejection has not been associated with inferior long-term outcomes
- Relative immunity from chronic rejection

Clinical Considerations
- Strategies Towards Tolerance
  - INDUCED
    - Specially designed immunosuppression, typically administered around the time of transplantation
  - SPONTANEOUS
    - “Standard” immunosuppression
Immunosuppression Withdrawal: Adult Liver Transplant Recipients

Adult Withdrawal Experiences

<table>
<thead>
<tr>
<th>Year</th>
<th>Center</th>
<th># Patients Attempted</th>
<th>“Tolerant” N (%)</th>
<th>Rejection</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acute</td>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>Mayo</td>
<td>12</td>
<td>0</td>
<td>6</td>
<td>3 (2 deaths)</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>Pittsburgh</td>
<td>95</td>
<td>18 (19%)</td>
<td>21</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>King’s</td>
<td>18</td>
<td>5 (17%)</td>
<td>13 (4 Bx Pr)</td>
<td>1 (1 retx)</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Spain</td>
<td>9</td>
<td>3 (33%)</td>
<td>6 (2 Bx Pr)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Miami</td>
<td>104</td>
<td>20 (19%)</td>
<td>70 (30 Bx Pr)</td>
<td>2 (1 retx)</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Ochsner</td>
<td>18</td>
<td>1 (6%)</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Tor Vergata</td>
<td>34</td>
<td>8 (23%)</td>
<td>26</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Ontario</td>
<td>26</td>
<td>2 (8%)</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>316</td>
<td>57 (18%)</td>
<td>168 (53%)</td>
<td>9 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

Spontaneous Tolerance: Risk of Immunosuppression Withdrawal

- Low likelihood of success: ~ 20%
- High risk of acute rejection: ~ 50%
  - “Readily” reversed
- Low risk of chronic rejection, graft loss, and death
- Uncertain benefit
  - Perhaps intuitive BUT
  - No convincing data

Changing the Risk - Benefit Analysis for Immunosuppression Withdrawal

- Biomarker(s) predictive of successful withdrawal
  - Enhance likelihood of success
- Biomarker(s) predictive of rejection
  - Enhance safety of withdrawal
- Demonstration of benefit
  - Medical, histologic, psychosocial, neurocognitive
Search for Biomarker(s) of Operational Tolerance

Withdraw IS

Failure: Rejection
[On IS]

Test Blood

Success: Tolerance
[Off IS]

PBMC Immunophenotyping:
Increased γδ T cells; Higher Vδ1/Vδ2 γδ T cell ratio

Higher % of γδ T cells among CD3+ T cells

Higher Vδ1/Vδ2 γδ T cell ratio

Martinez-Llordella et al. 2007

Li et al. 2004

Gene Expression Profiling:
Affymetrix Microarrays

SIGNSIFICANT ANALYSIS OF MICROARRAYS (SAM):
2367 genes (FDR<5%) TOL Non-TOL

Martinez-Llordella et al. JCI 2008

Gene Expression Profiles by QT-PCR

2D Principal Component Analysis on the basis of the expression of 33 genes differentially expressed between TOL and Non-TOL

Martinez-Llordella et al. JCI 2008; Sanchez-Fueyo, personal communication
Tolerance-associated Gene Expression: Functional Pathway Analysis

Prospective Validation Study: NCT00647283
Search for the Immunologic Signature of Operational Tolerance in Liver Transplantation

- Can BASELINE gene expression profile predict successful withdrawal?

<table>
<thead>
<tr>
<th>IS Withdrawal</th>
<th>Follow-up</th>
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<tbody>
<tr>
<td>6 – 9 months</td>
<td>12 months</td>
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</tbody>
</table>

Inclusion criteria:
1. Stable liver function >3 years after tx
2. Low dose IS
3. No rejection in previous 12 months
4. No autoimmune liver disease
5. Baseline liver biopsy

Trial Status: 2 / 2010

124 screened
102 enrolled
86 finished the study
8 Weaned (pending follow-up)

6 dropped-out

56 (65%) Non tolerant
30 (35%) Tolerant

Sanchez-Fueyo, personal communication

Prediction of Withdrawal Outcomes Using Baseline Blood Samples (58 recipients)

86
6 dropped-out

15% error rate
Sens: 0.53
Spec: 0.95
NPV: 0.85
PPV: 0.80

Fisher test 0.00011516

ATC 2009
Immunosuppression Withdrawal: Pediatric Liver Transplant Recipients

Pediatric Withdrawal Experiences

<table>
<thead>
<tr>
<th>Status / Outcome</th>
<th>Pittsburgh N = 64</th>
<th>Kyoto N = 591</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off drugs</td>
<td>22 (34%)</td>
<td>87 (15%)</td>
</tr>
<tr>
<td>Withdrawing</td>
<td>33 (52%)</td>
<td></td>
</tr>
<tr>
<td>Acute rejection</td>
<td>9 (14%)</td>
<td>1</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Mazariegos et al. 1997, 2007, & personal communication
Takatsuki, 2001; Oike, 2002; Li, 2004; Koshiba, 2009

Rationale for Clinical Trial of Immunosuppression Withdrawal for Pediatric Liver Transplant Recipients

- Lifelong burden of immunosuppression
- Higher likelihood of success
  - Previous withdrawal experiences
  - “Natural weaning” with growth
- Availability of parental living donor liver transplant recipients
  - Advantage of one haplotype HLA matching
  - Decreased ischemic injury
  - Donor material to support mechanistic studies

ITN029: Immunosuppression Withdrawal for Pediatric Parental Living Donor Liver Transplant Recipients

Single arm, three center pilot trial of 20 patients

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Estella Alonso, M.D.
Peter Whittington, M.D.
Steven Lobritto, M.D.
Jean Emond, M.D.
The Study Cohort
Inclusion / Exclusion Criteria

Deceased Donor

Withdrawal Schedule
Low dose module: 36 wks

High dose:
- Tacrolimus: ≥ 0.08 mg/kg/day
- Cyclosporine: ≥ 3.0 mg/kg/day

Each high dose "module": 8 wks

Primary Endpoint
Proportion of participants successfully withdrawn from immunosuppression, defined as those who remain off immunosuppression for at least one year with normal allograft function
ITN029: Outcomes

- 12 patients have met the primary endpoint and are operationally tolerant
  - Time off of immunosuppression: 20.4 – 41.1 months
- 8 patients did not meet the primary endpoint

ALT and GGT Profiles of Tolerant Participants

- All show NRH.
- First 2 show minimal focal portal inflammation.
- No change after weaning.

001-002

- 6/14/06 285 / 38 days
- 4/6/07 1101 / 854 days
- 6/30/09
All show NRH.

First f/u bx shows minimally increased portal inflammation but no damage.

Second f/u bx shows resolution of inflammation.

No change in fibrosis.

8 Participants Failed the Withdrawal Protocol

<table>
<thead>
<tr>
<th>Pt ID</th>
<th>Start (D)</th>
<th>Peak ALT / GGT</th>
<th>Biopsy Reading (Central Pathology)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>001013</td>
<td>35</td>
<td>121 / 244</td>
<td>ACR indeterminate</td>
<td>Baseline</td>
</tr>
<tr>
<td>002005</td>
<td>85</td>
<td>318 / 98</td>
<td>ACR moderate</td>
<td>IV + PO steroids + Escalation</td>
</tr>
<tr>
<td>001011</td>
<td>155</td>
<td>562 / 127</td>
<td>ACR indeterminate</td>
<td>Escalation</td>
</tr>
<tr>
<td>003002</td>
<td>169</td>
<td>312 / 197</td>
<td>ACR indeterminate biliary stricture</td>
<td>PO steroids + Escalation</td>
</tr>
<tr>
<td>001015</td>
<td>333</td>
<td>134 / 25</td>
<td>ACR indeterminate</td>
<td>Baseline</td>
</tr>
<tr>
<td>003001</td>
<td>NA</td>
<td>NA</td>
<td>ACR mild</td>
<td>Baseline</td>
</tr>
<tr>
<td>002008</td>
<td>NA</td>
<td>NA</td>
<td>ACR indeterminate</td>
<td>PO steroids + Escalation</td>
</tr>
</tbody>
</table>

Mild lymphocytic inflammation without tissue damage
**Failed Participants: Time of Failure Biopsies**

- ACR indeterminate
- ACR moderate

Increased severity and more broadly distributed inflammation with evidence of tissue damage

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**Clinical Predictors of Tolerance**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tolerant n = 12</th>
<th>Non-Tolerant n = 7</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) At Transplant</td>
<td>0.57 (0.32 - 2.43)</td>
<td>0.64 (0.44 - 7.48)</td>
<td>0.41</td>
</tr>
<tr>
<td>Age (years) At Study Entry</td>
<td>8.98 (5.22 - 12.14)</td>
<td>6.55 (5.03 - 15.27)</td>
<td>0.24</td>
</tr>
<tr>
<td>Male Gender</td>
<td>8 (66.7%)</td>
<td>3 (42.9%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Interval Between Transplant and Study Entry (years)</td>
<td>100.6 (53.5 - 138.7)</td>
<td>73.0 (52.7 - 93.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>History of Rejection</td>
<td>7 (58.3%)</td>
<td>4 (57.1%)</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Baseline ALT (U/mL)</td>
<td>31 (18 - 48)</td>
<td>30 (22 - 38)</td>
<td>0.85</td>
</tr>
<tr>
<td>Baseline GGT (U/mL)</td>
<td>27 (12 – 88)</td>
<td>16.0 (10 – 69)</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline Biopsy: Absence of Inflammation</td>
<td>11 (91.7%)</td>
<td>3 (42.9%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Histologic Predictors of Tolerance: Low C4d Stain Score**

- **TOLERANT**
- **NON-TOLERANT**

Histologic Predictors of Tolerance: High γδ1 / γδ2 T Cells in Portal Tracts

- **TOLERANT**
- **NON-TOLERANT**

*Days after OLTx* vs *Total C4d Score*

*Days after OLTx* vs *Delta 2+ / CD3+

*Days after OLTx* vs *Delta 1+ / CD3*
U34/U01 Clinical Trial (NIDDK)

- Prospective, randomized, multi-center trial to assess safety and efficacy of immunosuppression withdrawal
- Planned enrollment: 108 participants
  - 3:1 Withdrawal : No withdrawal
- Include recipients of deceased donor liver transplants
- Primary endpoint: stable allograft histology and liver test profiles one year off of immunosuppression

Immunosuppression Withdrawal: Perhaps No Longer Hit or Miss

- Immunosuppression withdrawal has been attempted in liver transplant recipients with modest success.
  - Children more likely to succeed than adults.
- Although allograft dysfunction/rejection does occur, experience suggests that attempted withdrawal, at least in the clinical trial setting with intensive monitoring, is safe.
- The pilot pediatric trial (ITN029ST) has also shown that:
  - Spontaneous tolerance appears, thus far, to be durable. Concurrent illnesses including infections has not broken tolerance.
  - The absence of immunosuppression has not led to deterioration of allograft histology.
  - Some clinical parameters may hold promise to predict tolerance.

But Perhaps Still Unwise?

- Currently, data confirming the intuitive benefit of immunosuppression withdrawal is lacking.
- There is also insufficient data to be confident that the absence of immunosuppression does not lead to deterioration of allograft histology.
- The identification of biomarker(s) – clinical, histologic, immunologic, and/or molecular – that can accurately predict successful withdrawal would substantially mitigate the risk of withdrawal.
- Additional large, carefully designed trials with adequate follow-up are required.