Tolerance Induction in Transplantation

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Cadaveric Renal Allograft Survival
“Better living through pharmacology?”

- Radiation
- Prednisone
- 6-MP
- Cyclosporine Emulsion
- Tacrolimus
- MMF
- Daclizumab
- Basiliximab
- Sirolimus
- Alemtuzumab

Rejection <12 mo
1 Year Survival

Percent

'60  '65  '70  '75  '80  '85  '90  '95  '00  '05
Causes of Graft Loss

Causes of Late Graft Loss

- Glomerulonephritis: 6%
- Other: 5%
- Chronic rejection: 36%
- Death with function: 50%

Causes of Death with Function

- Other: 10%
- Cardiovascular: 36%
- Unknown: 17%
- Accident/suicide: 2%
- GI tract disorder: 2%
- Stroke: 6%
- Malignancy: 9%
- Infection/sepsis: 18%

Immunosuppression: Limitations

- inadequate efficacy
  - acute and chronic rejection
  - T1/2- 14 yrs
- non-specific
  - infection
  - cancer
- Medication side effects
  - High blood pressure
  - Elevated cholesterol
  - Hirsuitism
  - Moon Facies
  - Gum hyperplasia
- Cost- $10,000-15,000/yr

Why Tolerance?

- Long-term results are static
- Chronic Rejection is still a problem
- Side-effects of chronic Immunosuppression (infection, cancer, CVD, …)
Mechanism of Tolerance

- **Deletion**
  - Central (in thymus)
  - Peripheral
    - AICD (active T cell death)
    - Passive cell death
    - Anti-T cell treatment

- **Anergy**

- **Immune deviation**
  - Th1 (IL-2, IFN-γ) Delayed Type Hypersensitivity (rejection)
  - Th2 (IL-4, IL-10) B cells, Tr1 cells (acceptance)
  - Th17 (IL-17A-F, TGF-β, IL-6) Autoimmunity (rejection)

- **Suppression**
  - T<sub>REG</sub> cells (CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup>)
  - Other (Tr1, CD8, CD4<sup>+</sup>CD8<sup>+</sup>, etc)
Identification of “tolerance genes” in training- and test-set patient samples.

- 17 tolerant pts
- 33 genes
- More FoxP3
- TGF-b
- Co-stimulation molecules
- IL-2 related genes

Brouard S et al. PNAS 2007;104:15448-15453
The Presence of Foxp3 Expressing T Cells Within Grafts of Tolerant Human Liver Transplant Recipients

Transplantation 2008; 86:837-843
Tolerance Induction from Rodents to Large animals
Sir Peter Medawar
Nobel Prize in Physiology or Medicine 1960
1) Recipient B10
   950 R (Cs source)

2) Mix
   Syngeneic bone marrow
   T cell (-)
   Allogeneic bone marrow

3) Skin Grafting
   Recipient B10
   Donor B10.D2
Non-Lethal Preparative regimen

1. Anti CD4 mAb
2. Total Body Irradiation (TBI) 300 R
3. Thymic Irradiation 700 R
4. Donor Bone Marrow B10.D2

Mixed Chimerism

Skin Grafting
Table 1
Methods that have induced transplantation tolerance

<table>
<thead>
<tr>
<th>Method</th>
<th>Mice</th>
<th>Primates and humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhancement</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DST</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Peptides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-MHC mAB’s</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ALS</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-CD24</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-CD25</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Total lymphoid irradiation</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Anti-CD3 toxin</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Costimulatory blockade</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Chimerism</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

ALS, antilymphocyte serum; DST, donor-specific transfusion.
Standard Regimen

TBI 1.5X2

Kidney Tx
DBM
Splenectomy

ATG

-6 -5
-2 -1 0
28 days

CYA
Mixed Chimerism in Monkeys

![Graph showing mixed chimerism in monkeys with data points for lymphocytes, monocytes, and GRN over days post-transplant.](image)
M393
28 days after Skin Tx
(362 days after Kidney Tx)
Renal Allograft Biopsy at 10 years
Tolerance Induction in Clinical Transplantation
Induction Of Kidney Allograft Tolerance After Transient Lymphohematopoietic Chimerism In Patients With Multiple Myeloma And End-Stage Renal Disease
Non-Myeloablative Regimen

- Thymic Irradiation
  - 7 Gy
- Cyclophosphamide
- Kidney Tx
- Donor Bone Marrow Tx
- Anti-CD2 mAb

Timeline:
- Day -7
- Day -5
- Day -4
- Day -2
- Day -1
- Day 0
- Day 1

Duration: 9-14 months
Revised Regimen

Exclude patients with positive PRA

Thymic Irradiation
7 Gy

Kidney Tx

Donor Bone Marrow Tx

Cyclophosphamide

-7 -5 -4

Thymic Irradiation 7 Gy

anti-CD2 mAb

-2 -1 0 1 day

steroids

CYA

9-12 months

Rituximab (anti-B cell)
## Withdrawal of Immunosuppression

<table>
<thead>
<tr>
<th>Graft Survival</th>
<th>Immunosuppression</th>
<th>Allo-antibody</th>
<th>C4d</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 &gt; 6.3 yrs</td>
<td>OFF (&gt; 5.5 yrs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#2 &gt; 5.5 yrs</td>
<td>OFF (&gt; 4.3 yrs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#3 10 days</td>
<td>-</td>
<td>Anti-HLA class I and II 10 days after D/C drugs</td>
<td>-</td>
</tr>
<tr>
<td>#4 &gt; 3.8 yrs</td>
<td>OFF (&gt; 3 yrs)</td>
<td>Anti-HLA class II 1 mo after D/C drugs</td>
<td>+</td>
</tr>
<tr>
<td>#5 &gt; 2.8 yrs</td>
<td>OFF (&gt; 2 yrs)</td>
<td>Anti-HLA class II 1 year after D/C drugs</td>
<td>-</td>
</tr>
</tbody>
</table>
Multi-Lineage Chimerism

Day 7

Day 14
**Neutrophil Counts**

**Lymphocyte Counts**

*weeks/months after transplant*
Current Problems to Overcome

- Cytokine Syndrome
- Acute Humoral Rejection
- Production of anti-donor antibody
## Complications

<table>
<thead>
<tr>
<th></th>
<th>Cytokine Syndrome</th>
<th>Acute Humoral Rejection</th>
<th>Anti-Donor Antibody</th>
<th>Long-term Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>normal</td>
</tr>
<tr>
<td>#2</td>
<td>+</td>
<td>Day 45, reversed</td>
<td>-</td>
<td>normal</td>
</tr>
<tr>
<td>#3</td>
<td>+</td>
<td>Day 10, Graft loss</td>
<td>Anti-HLA class I and II</td>
<td>Re-transplant</td>
</tr>
<tr>
<td>#4</td>
<td>+/-</td>
<td>-</td>
<td>Anti-HLA class II (1 mo after D/C IS)</td>
<td>C4d+, normal</td>
</tr>
<tr>
<td>#5</td>
<td>+</td>
<td>Day 10, reversed</td>
<td>Anti-HLA Class II (1 yr after D/C IS)</td>
<td>C4d-, normal</td>
</tr>
</tbody>
</table>
MECHANISM OF TOLERANCE (HYPOTHESIS)

Donor Bone Marrow Transplantation

Transient Chimerism

Induction of Regulatory (Suppressor) cells (CD25+CD4+, Foxp3+)
New Regimen

Exclude patients with positive PRA

Kidney Tx
Donor Bone Marrow Tx

Thymic Irradiation
7 Gy

Cyclophosphamide

-7 -5 -4

-2 -1 0 1 5

12 day 9-12 months

Anti-CD2 mAb

Rituximab (anti-B cell)

steroids

FK

Massachusetts General Hospital
<table>
<thead>
<tr>
<th></th>
<th>TOL</th>
<th>Stable on IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>IS meds</td>
<td>0</td>
<td>Triple-16/18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Double-2/18</td>
</tr>
<tr>
<td>Post-Tx DM</td>
<td>0</td>
<td>2/18 (11%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HTN</td>
<td>0</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>Anti-Hyperlipid</td>
<td>0</td>
<td>4/18 (22%)</td>
</tr>
<tr>
<td>PPI</td>
<td>0</td>
<td>14/18 (78%)</td>
</tr>
</tbody>
</table>
## Maintenance Cost
### Tolerance vs. Stable on IS

<table>
<thead>
<tr>
<th>Medication</th>
<th>TOL</th>
<th>Stable on IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prograf (4mg/day)</td>
<td>0</td>
<td>$5,342</td>
</tr>
<tr>
<td>MMF (1.5g/day)</td>
<td>0</td>
<td>$6,048</td>
</tr>
<tr>
<td>Prednisone (5mg/day)</td>
<td>0</td>
<td>$ 120</td>
</tr>
<tr>
<td>Nexium (20mg/day)</td>
<td>0</td>
<td>$1,836</td>
</tr>
<tr>
<td>Lipitor (10mg/day)</td>
<td>0</td>
<td>$ 983</td>
</tr>
<tr>
<td>Atenolol (50mg/day)</td>
<td>0</td>
<td>$ 56</td>
</tr>
</tbody>
</table>

| Annual maintenance Cost | 0 | $ 14,385 |

*Note: Costs are in USD.*
Delayed Tolerance

Donation from deceased donor (organ and Bone marrow)

1) Solid organ Tx

Conditioning

Cryopreservation of BM cells

2) Thaw and BMT

Conditioning
Memory Cells

Tolerance Induction Using Hematopoietic-Stem Cell : Stanford Protocol

- post-transplantation conditioning regimen of total lymphoid irradiation (800-1200 cGY), HPSC and antithymocyte globulin.
- 15 pts, HLA-matched LDKT, CYA for 6 months
- 8 pts came off IS
- persistent mixed chimerism
- F/U 28 m

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Northwestern Protocol

- 11 HLA-mismatched LDKT
- Fludarabine, cyclophosphamide, 200 cGy TBI days -4 to -1
- CD34+ HSC on day +1
- FK, MMF for 1 year
- 9 pts developed mixed chimerism
- 7 pts started the weaning, One totally off
- One graft loss, need re-TX
**IMMUNOLOGIC**
- Large cellular compartment
  - Hematopoietic regulators (γδ T, NK/NKT cells, pDC)
  - Dilutional mass effect
- Regulatory proteins/cytokines
- Alloantibody dissolution
- Mixed hematopoietic microchimerism

**CLINICAL**
- Low relevance of acute rejection
- Rare chronic rejection
- Low immunosuppression required
- Regulatory effect of combined liver-other organ
- Insignificance of HLA match
Recent and Ongoing Clinical Trials of Immune Tolerance Therapies

- Kidney transplant Alemtuzumab, a tacrolimusb for 60 d, sirolimus (rapamycin) for 1 y
- Kidney transplant Daclizumab, LEA29Y (belatacept), MMF, sirolimus for 1 y
- Kidney transplant Alemtuzumab, donor stem-cell infusion, tacrolimus/sirolimus and MMF for 1 y
- Kidney & bone marrow transplant
  - Cyclophosphamide, MEDI-507 (siplizumab), 7 Gy thymic irradiation, donor bone marrow, cyclosporine for 60 d
- Kidney & bone marrow transplant for myeloma patients
  - Cyclophosphamide, ATG, 7 Gy thymic irradiation, donor bone marrow, cyclosporine for 36-60 d
- Liver transplant Alemtuzumab, tacrolimus slowly withdrawn
- Liver transplant Gradual withdrawal of maintenance immunosuppression in patients who underwent living donor liver transplants as children
- Liver transplant Alemtuzumab vs 3 mo of steroids followed by tacrolimus slowly withdrawn
Barriers to Tolerance Induction

• Thymic and peripheral T cell-mediated alloresistance
• Donor-specific antibodies
• Heterologous immunity
• Non-immunological tissue damage

SPECIFIC HURDLES

chimerism

tolerance

toxicity of host conditioning

clone size

memory cells

homeostatic proliferation

heterologous immunity

concomitant infections

solid organ

stem cells

risk of GVHD

recipient
donor
Future Directions

1- Extend the applications

2- Memory cells

3- T regs

4- Tolerance assays
Thank You