Why so Sensitive? Desensitizing Protocols for Living Donor Kidney Transplantation

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Objectives of this Talk

- Define the “sensitized” patient
- Describe the scope of the problem for a “sensitized” patient to undergo successful living donor transplantation
- Describe the process to identify immunologic barriers to living donor transplantation
- Describe the current UCSF protocol for desensitization prior to living donor transplantation

What is a “Sensitized” Patient?

- Any patient with detectable anti-HLA antibodies
- This state of sensitization is defined by the patient’s PRA (panel reactive antibody) level
- PRA ranges from 0% (∾) to 100% (⋯)
- Defines the likelihood of finding a compatible donor from the general population

How Do We Become “Sensitized”?

- Blood transfusions
- Pregnancy
- Previous Transplants
- Infections
Human Leukocyte Antigen System

- Name of the major histocompatibility complex (MHC) in humans
- Genes on chromosome 6 produce cell surface molecules (antigens)
- HLA corresponding to MHC Class I are the A, B and C antigens and are expressed on all nucleated cells in the body
- HLA corresponding to MHC Class II are the DR, DQ and DP antigens and are expressed on restricted cells such as B lymphocytes, activated but not resting T lymphocytes, endothelial cells and antigen presenting cells
- Molecular typing can identify the antigens in an individual
- Commonly the A, B and DR antigens are reported
- The antigens are co-dominantly expressed so 2 A, 2 B and 2 DR antigens for a total of 6 expressed antigens are reported

How Do We Detect anti-HLA Antibodies?

Cytotoxicity Assays for Antibodies

- Viable T or B Lymphocytes
- Cells from a different individual to each well
- Patient serum to every well
- All Cytotoxic Antibodies Detected
  - IgG and IgM
  - HLA
  - Autoantibodies
  - Non-HLA alloantibodies
- 6/56 Positive
- 11% PRA

Microparticle Arrays

- HLA Bound to Microparticles
- Labeled Antihuman Globulin
- Fluorescence Detection
- 31/55 Positive
- 56% PRA
Why Do We Care about anti-HLA Antibodies?

Scope of the Problem

- Presence of anti-HLA antibodies impair the ability to find a compatible donor and diminish the long term post-transplant outcomes
- 40-50% of patients have detectable anti-HLA antibodies prior to transplantation
- Waiting times for transplant increase as the anti-HLA antibody levels increase
- Patients with potential living donors can be excluded from transplant due to incompatibility from anti-donor antibodies
Eight-year grafts survival analysis of kidney transplants in relation to DSA status.

Approach to the “Sensitized” Patient for Successful Living Donor Transplantation

- Characterize the immunologic status of the recipient
- Perform detailed crossmatch procedures between the recipient and the donor
- Determine whether a “positive” crossmatch can be manipulated to have transplantation performed safely and successfully

Histocompatibility Meeting

- Meet bi-weekly to review HLA and crossmatch data, order extended testing, identify candidates for desensitization protocols and participation in the paired kidney exchange
- Dr. Lee Ann Baxter-Lowe, ITL Lab Director
- Transplant Nephrologists
- Living Donor Transplant Coordinators
  - Diane Mak-Kaplan, Diana Zographos, Sarah McIndoe

Immunogenetic Testing between Recipient & Donor

- Cytotoxicity Crossmatch
  - T cell
  - B cell
- Flow Cytometry Crossmatch
  - T cell (" +" if FCXM > 19 channels)
  - B cell (" +" if FCXM > 39 channels)
- Determination of PRA Class I & II (%)
- Identification of specificity of recipient’s antibody
  - anti-HLA antibodies to the general population and donor specific anti-HLA antibodies (DSAs)
Definitions

- **Positive Cytotoxic Crossmatch**: detectable anti-donor HLA antibodies that cause cell death
- **Positive Flow Cytometry Crossmatch**: detectable anti-donor HLA antibodies that do not cause cell death
Factors that Contribute to a Positive Flow Cytometry Crossmatch and Donor Specific Antibodies

• Panel Reactive Antibody Level > 30%
• Received blood transfusion or previous organ transplant
• History of multiple pregnancies
• Current donor is related to previous donor
• Donor is child to mother
• Donor is husband to wife

Options for the Patient with a Positive Flow Cytometry Crossmatch

• Paired Exchange or National Kidney Registry
  - Is there a living donor that can be exchanged with another pair?
• Desensitization
  - Need to profile the characteristics of the recipient’s immunologic state
• Wait on the Deceased Donor Wait List

The Sensitized Patient with a Potential Living Donor

• Advantage of time
• Ability to run more detailed analysis of the donor specificity of the recipient’s antibody
• Ability to repeatedly monitor response to intervention

Desensitization Approaches

Anchor Therapies

• Intravenous Immunoglobulin (IVIg)
• Plasmapheresis

Enhancers

• Splenectomy
• Anti-CD20 Antibody (Rituxamab)
• Bortezomib
• Anti-Lymphocyte Globulin Induction
• Tacrolimus, Mycophenolate mofetil and Prednisone
**Intravenous Immunoglobulin (IVIg)**

- **What is it?**
  - An immune modulating therapy made from pooled human blood plasma
  - Therapy for inflammatory and immune system disorders since late 1980s
  - Half life 21-28 days

- **Why is it used in transplant recipients?**
  - Reduces anti-HLA antibody levels, interferes with existing antibodies to cause injury to the kidney transplant as well as preventing the antibodies from returning
  - Can allow transplant to occur in the face of a positive cross-match
  - Can result in fewer rejection episodes post-transplant and more successful long-term graft outcomes

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**Profile of IVIg Products**

<table>
<thead>
<tr>
<th>Name</th>
<th>GAMMA-IVS 10%</th>
<th>GAMUNEX</th>
<th>CARDUNEX</th>
<th>GAMMA-IVS 15%</th>
<th>Polygammablobulin</th>
<th>Panglobulin</th>
<th>Hyperglobulin</th>
<th>Immunegam</th>
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<td>MFR</td>
<td>Aventis</td>
<td>Bayer</td>
<td>ZLB</td>
<td>Baxter</td>
<td>American Red Cross</td>
<td>American Red Cross</td>
<td>Grifols</td>
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<td>Sugar</td>
<td>Sucrose</td>
<td>None</td>
<td>Sucrose</td>
<td>Glucose</td>
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<td>Glucose</td>
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</table>
|**Gamunex is the preferred IVIg product for desensitization in transplant recipients**

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**Gamunex Administration**

- Gamunex 2 gm/kg IV divided into two infusions
  - Days 4 and 2 before the transplant date
  - Infusion rate approx 1 hour: 10 gm IVIG

Pre-medications:
1. Tylenol
2. Benadryl
3. Hydrocortisone

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**Side Effects**

- **Infusion related:**
  - Flu-like symptoms: headache, fever, fatigue, chills, flushing, nausea and vomiting
  - Anaphylaxis

- **Rare:**
  - Thrombotic Event
  - Hemolytic Anemia
UCSF Desensitization Protocol

- **IVIG 10-day Protocol**
  - involves IVIg and pretransplant immunosuppression

IVIG 10 Day Protocol

Crossmatch Results Criteria:
- T/B Cell Cytotoxic Negative with a T/B FACS Positive and an identifiable donor specific antibody (DSA)

Inclusion Criteria:
- Age 18-70 otherwise medically and surgically suitable for transplantation
- T and B cell cytotoxic negative crossmatch
- Positive T and/or B Flow Cytometry crossmatch
- Identifiable donor specific antibody

Exclusion:
- Inability to tolerate IVIG
- Active Cardiovascular Ischemia

UCSF Protocol for Cytotoxic Negative/FACS Flow Positive Living Renal TX

UCSF Outcomes Following a 10 Day IVIg Desensitization Protocol

Mycophenolate mofetil (also on Protonix)
Tacrolimus
IVIg ADMINISTRATION (2 gm/kg IV – may be split over 2 doses on pre-Tx Day 4 & Day 2)
### Demographics

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<th>Age</th>
<th>46.24 +/- 13.18</th>
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<tbody>
<tr>
<td>Sex</td>
<td>30/39 (77%) female</td>
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<tr>
<td>ESRD Etiology (y/n)*</td>
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</tr>
<tr>
<td>Diabetes</td>
<td>8%</td>
</tr>
<tr>
<td>HTN</td>
<td>31%</td>
</tr>
<tr>
<td>ADPKD</td>
<td>8%</td>
</tr>
<tr>
<td>Lupus</td>
<td>13%</td>
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<tr>
<td>FSGS</td>
<td>8%</td>
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<tr>
<td>Glomerulonephritis</td>
<td>28%</td>
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<tr>
<td>Other</td>
<td>18%</td>
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<tr>
<td>Unknown</td>
<td>8%</td>
</tr>
<tr>
<td>Sensitization History</td>
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<tr>
<td>Known Prior Pregnancies</td>
<td>57% women</td>
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<tr>
<td>Prior Transplant</td>
<td>51%</td>
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<tr>
<td>Unknown</td>
<td>23%</td>
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<tr>
<td>Patients meeting criteria for desensitization</td>
<td>25/39 (64%)</td>
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<td>Historical Positive Cross-matches</td>
<td>9/39 (23%)</td>
</tr>
<tr>
<td>&quot;Fuzzy Criteria&quot;</td>
<td>5/39 (13%)</td>
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### Immunologic Profile

| Class I PRA >30% | 68% |
| Class I PRA > 80% | 13% |
| Class II PRA >30% | 75% |
| Class II > 80% | 29% |
| T Cell FCXM positive | 52% |
| B Cell FCXM positive | 68% |
| T and B cell FCXM positive | 20% |
| Class I DSA | 68% |
| Class II DSA | 52% |
| Class I and II DSA | 20% |

### Outcomes

| Mean nadir creatinine | 1.0 |
| Graft losses          | 2/25 (8%) |
| Mean current creatinine of those with functioning graft | 1.5 |
| Urine protein/creatinine | 0.5 |
| Mean days of follow-up | 775 (SD ± 470) |

- One graft loss due to presumed recurrent FSGS
- One graft loss due to Recurrent primary oxalosis
- No graft losses due to rejection

### Explanations for deviations from protocol

- Our “desensitization” protocols were developing in parallel with the technology to support the protocol
  - B cell Flow Cytometry
  - Cutoffs for truly positive and truly negative were not clearly established
  - Single Antigen bead assays became available in the midst of protocol development and implementation
- Other centers were reporting successes, and later failures with desensitization
Other Pre-Transplant Desensitization Protocols in the United States

- Addition of Rituximab (antiCD20 monoclonal antibody) to IVIg (Cedars-Sinai)
- Addition of Plasmapheresis to actively remove existing anti-HLA antibodies (Johns Hopkins)
- Addition of Bortezomib (proteosome inhibitor) (Mayo Clinic and U of Cincinnati)

Summary

- Living donation is the preferred treatment for patients requiring transplantation
- Thorough immunologic investigation of the donor-recipient pair is needed to ensure optimal outcomes
- Desensitization protocols utilizing IVIg can safely allow previously incompatible transplants to occur
- Further protocols incorporating new agents may expand the number of patients receiving transplants from incompatible donors