Antibody Response in Kidney Transplant Recipients

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Allograft rejection: a historical perspective

• Mid 1950’s: first successful solid organ (kidney) transplant between identical twins
  – Transplants between non-HLA identical individuals continue to rapidly reject without immunosuppression

• 1960’s: Antibodies to HLA antigens identified as major predictors of rapid (hyperacute) graft loss
  – Cytotoxic crossmatch assays developed by Terasaki et al. all nearly eliminate hyperacute AMR

• 1970’s-1990’s: with early AMR “cured”, focus is on T-cell mediated rejection
  – Introduction of modern immunosuppression (steroids, antiproliferatives, CNIs, induction) drops cellular rejection rates from 80% to ~10%

• Late ‘90’s-current: with cellular rejection nearly “cured”, Ab-mediated graft damage re-recognized as an important entity in graft outcome
  – New highly sensitive Ab-detection techniques continue to improve
Outline

• Antibody production: basic B-cell biology
• Preexisting DSA:
  – Pre-transplant cross matching techniques
  – Preliminary data from our center’s experience with pre-transplant DSA and outcomes
• De novo (post-transplant) DSA:
  – Results of a retrospective analysis of our post-transplant DSA screening protocol
B-cell biology

- Considered innate immunity
- Produce antibodies with low affinity to antigen (microbial structures)

- Adaptive immunity
- Antibody production with high affinity for antigen encountered in blood and peripheral lymphoid organs
Immature B cells migrate to secondary lymphoid tissue and await antigen presentation
In organ transplant the antibodies of interest are directed against another individual’s MHC antigens

- When does the immune system encounter another individual’s MHC peptides?
  - Organ transplant
  - Transfusions
  - Pregnancy
- Antibody production requires collaboration between B and T cells, APCs

- Differentiation:
  - CD8 cytotoxic T cells
  - CD4 helper T cells
    - Th1/Th2/Th17/Treg

- Migration to secondary lymph organs via circulation
B cell maturation occurs via Th-derived cytokines

- B cells take up antigen from APC and display fragments via surface MHC II, migrate to boundary of the B and T cell zone.
- This allows Th cells to identify appropriate B cells targets.
  - Cytokines (IL-2, IL-4) and costimulatory molecules (CD40-CD40L) stimulate B cell maturation → rapid mutation and proliferation.
  - Selection of clones displaying IgG with high affinity for the donor MHC antigen being presented results in populations of 1) plasma cells producing Abs with high affinity for MHC and 2) memory B cells.
Antibody binds mismatched MHC antigen on donor cells, fixes complement → cell death
Summary and big picture

• The MHC complex is a region of highly polymorphic genes that has evolved over millions of years in response to the constant threat of microbial invasion
  – This is beneficial for vertebrates that wish to ensure the continuity of their species in the presence of pandemic infection
  – This is problematic for transplant patients and physicians who wish to transfer a functioning organ from one individual to another
    • MHC gene products (HLA antigens) are the principal antigenic determinants of graft rejection

• The B cell response to HLA antigens involves collaboration with APCs and T cells that recognize foreign MHC
  – Activated B cells with high affinity to Ag survive as memory or Ab-producing plasma cells

• HLA Ab binding to donor MHC Ag results in graft injury via complement
  – Manifests clinically as hyper-acute, acute, or chronic AMR
Measured PRA and final XM via complement dependent cytotoxicity (CDC)(1960’s)

- PRA predicts likelihood of positive final crossmatch with any given donor from the general population.
Flow Crossmatch (FCXM) (1980s)

- +FCXM using T-cells suggests class I Abs (HLA-A, B, or C)
- +FXCM using B-cells suggests class II Abs (HLA-DR, DP, or DQ)

Bray RA et al. Imm Research 2004; 29/1-3: 41-53
Single Antigen Beads (SAB): 2000’s
More sensitive, *Abs to exact antigens defined*
SAB report allows identification of DSA and non-DSA HLA Abs
• What are the clinical implications of pre-transplant DSA screening tests (ie, who should be desensitized?)
  – CDC XM +: Highest risk for rejection
    Contraindicated without desensitization
    High risk without desensitization
  – DSA detected by SAB: ??
Patients with pre-existing DSA at the time of transplant experience worse graft outcomes

- 334 consecutive low risk (PRA <20%) kidney recipients 1999-2004
  - CDC (-), no FCXM done. Tested retrospectively for pre-transplant DSA
  - No thymoglobulin induction. CNI-based immunosuppression in 80%, SRL-based in 20%.

How many of these patients would have had a positive FCXM?

Amico et al. Transplantation 2009; 87: 1681–1688
Should we avoid transplanting all patients with pre-existing DSA?

• Not all DSA detected by SAB are strong enough to result in a positive FCXM test

• **Hypothesis:** In patients with pre-transplant DSA, those with positive FCXM experience worse outcomes vs. those with negative FCXM

• Retrospective analysis of all kidney transplants 9/07 to 9/09
  – Per protocol, all kidney/SPK recipients undergo FCXM at the time of transplant and SAB analysis at the time of or within 3 months of transplant.
  – Historic (stored) sera up to 6 months prior is also tested by FCXM and SAB analysis when available.
Graft outcomes by pre-transplant antibody status

<table>
<thead>
<tr>
<th></th>
<th>FXCM+</th>
<th>FCXM-/DSA+</th>
<th>FCXM-/DSA-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (263)</td>
<td>12</td>
<td>33</td>
<td>218</td>
</tr>
</tbody>
</table>

**Acute Rejection**
- FXCM+: 42%
- DSA+: 21%
- Negative XM: 13%
- RR: 3.13 (1.48-6.63), p=0.003
- RR 1.59 (0.76-3.34), p=0.22

**Graft Loss**
- FXCM+: 25%
- DSA+: 3%
- Negative XM: 6%
- RR: 4.58 (1.30-16.07), p=0.018

**2 yr MDRD GFR (cc/min)**
- FXCM+: 49
- DSA+: 65
- Negative XM: 61
Pre-transplant management of DSA

FCXM+ (n=12)
- 5/12 = induction + IVIG
  - AR in 1/5 (20%)
- 7/12 = induction, no IVIG
  - AR in 4/7 (57%)

FCXM-, DSA+ (n=33)
- 31/33 received induction
  - AR in 6/31 (19%)
- 2/33 no induction
  - AR in 1/2
Proposed algorithm based on screening results

Sensitized patient with living or deceased donor

(Pre-transplant HLA-Ab screening)

- All testing negative
  - 10%
  - Induction per center protocol

- FCXM (-) DSA (SAB) (+)
  - 20%
  - Induction: r-ATG (+/- IVIG if high risk?)

- FCXM (+) DSA (SAB) (+)
  - 40-60%
  - Desensitize +/- Paired donation

- CDC XM (+)
  - 60-80%
  - Contraindicated

AR Risk
Conclusions: pre-transplant DSA

- Using data from both FCXM and SAB assays we are able to more accurately characterize immunologic risk in recipients with DSA.

- Patients with DSA and positive FCXM are at risk (40-60%) of AR and desensitization is indicated.

- DSA alone (negative FCXM) may be associated with a small increased AR risk
  - r-ATG induction for living or deceased donor transplant.
Post-transplant DSA have been associated with worse graft outcomes

- Multiple studies have shown association and predictive value of post-tx DSA in both acute rejection (AR) and chronic allograft dysfunction

- While these studies have established an association between DSA and graft failure, they do not offer info regarding clinical situation in which DSA was formed.

- A prospective DSA screening protocol has allowed us to more accurately characterize de novo DSA development

Terasaki et al. AJT 2007; 7: 408–415
Goals and Methods

• We analyzed data from a prospective post-Tx DSA screening protocol in 244 patients consecutively transplanted between 9/07 and 9/09 without pre-existing DSA to study:
  • Timing and clinical impact of de novo DSA production
  • Impact of DSA strength and class on graft outcomes.

• All kidney and kidney/pancreas recipients were screened for DSA (A, B, Cw, DR, DQ, and DP) using single antigen beads (Luminex) at 1, 6, 12, and 24 months post-transplant and when clinically indicated (decline in renal function).
  • Normalized MFI > 500 considered positive.
## Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients N (%)</th>
<th>DSA(+) N (%)</th>
<th>DSA(-) N (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>244</td>
<td>65 (27)</td>
<td>179 (73)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (Mean) ± SD</strong></td>
<td>49 ± 13.6</td>
<td>51 ± 12.4</td>
<td>44 ± 12.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Donor Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>• Deceased</td>
<td>138 (57)</td>
<td>39 (60)</td>
<td>99 (55)</td>
<td>.56</td>
</tr>
<tr>
<td>• Living</td>
<td>106 (43)</td>
<td>26 (40)</td>
<td>80 (45)</td>
<td></td>
</tr>
<tr>
<td><strong>HLA mismatch (Mean) ± SD</strong></td>
<td>3.6 ± 2.5</td>
<td>4.0 ± 1.3</td>
<td>3.4 ± 1.7</td>
<td>.01</td>
</tr>
<tr>
<td><strong>PRA (Mean) ± SD</strong></td>
<td>9.3 ± 22.3</td>
<td>7.6 ± 16.8</td>
<td>9.9 ± 23.0</td>
<td>.46</td>
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<tr>
<td><strong>Induction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• None</td>
<td>71 (29)</td>
<td>18 (28)</td>
<td>53 (30)</td>
<td>.73</td>
</tr>
<tr>
<td>• Thymoglobulin</td>
<td>162 (66)</td>
<td>43 (66)</td>
<td>119 (66)</td>
<td></td>
</tr>
<tr>
<td>• IL2-RA</td>
<td>11 (5)</td>
<td>4 (6)</td>
<td>7 (4)</td>
<td></td>
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<tr>
<td><strong>Immunosuppression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Tac/MPA/Pred</td>
<td>212 (87)</td>
<td>50 (77)</td>
<td>162 (90)</td>
<td>.01</td>
</tr>
<tr>
<td>• SRL/MPA/Pred</td>
<td>16 (6.5)</td>
<td>6 (9)</td>
<td>10 (6)</td>
<td></td>
</tr>
<tr>
<td>• Other</td>
<td>16 (6.5)</td>
<td>9 (14)</td>
<td>7 (4)</td>
<td></td>
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</tbody>
</table>
New DSA is detected by protocol in 52/65 DSA(+) patients, with 90% detected in first 6 months
Acute rejection is more likely to occur in DSA (+) patients

<table>
<thead>
<tr>
<th></th>
<th>All Patients n=244</th>
<th>DSA(-) n=179</th>
<th>All DSA(+) n=65</th>
<th>Protocol DSA (+) n=52</th>
<th>Non-protocol DSA(+) n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Rejection (%)</td>
<td>36 (15)</td>
<td>17 (9.5)</td>
<td>19 (29)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 (19)</td>
<td>9 (69)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>• AMR (%)</td>
<td>5 (2)</td>
<td>0</td>
<td>5 (8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 (23)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Cellular (%)</td>
<td>31 (13)</td>
<td>17 (9.5)</td>
<td>14 (22)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 (15)</td>
<td>6 (46)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p≤.001 compared to no DSA, <sup>b</sup>p<.05 compared to no DSA
DSA was detected prior to rejection episode in only 3/19 patients

X: Cellular Rejection
O: Antibody Rejection
>: DSA detection

DSA detected by indication screening
DSA detected by protocol screening

Patients with rejection and DSA

DSA detected prior to rejection

Days Post-Transplant
Prior acute rejection episodes account for higher graft failure rates in patients with DSA

- In patients with both DSA and AR, most DSA was detected after the AR episode.
- These data suggest increased graft failure rates in patients with post-transplant DSA are due to previous AR episodes rather than DSA alone.
Is acute rejection alone responsible for graft loss? Does DSA matter at all?

- These data suggest the *combined* insult of acute rejection and de novo DSA production is an important predictor of graft survival.
Development of both HLA class I and II DSA is associated with graft failure.
MFI levels >6000 are associated with worse outcomes

A: MFI >1000 vs. <1000

B: MFI >3000 vs. <3000

C: MFI >6000 vs. <6000

D: MFI >6000 vs. <6000, no AR

Graft Survival

Time (Months)

p=.21

p=.026

91%

67%

Graft Survival

Graft Survival

Graft Survival

Graft Survival

p=.35

91%

67%
Conclusions: post-transplant DSA

• A prospective screening protocol detected DSA in 27% of kidney and SPK recipients, majority of which detected within the first 6 months of transplant.

• Worse graft survival in DSA(+) patients is due to prior AR episodes in combination with concurrent/subsequent DSA production as opposed to the presence of DSA or AR alone.

• Combined development of both HLA class I and II DSA and MFI values > 6000 in patients with AR are associated with graft failure.

• Ongoing follow up will help to define the clinical implications of post-tx de novo DSA in the otherwise stable transplant patient.
Thank You

Questions/comments?