HLA Antibody Identification Procedures and How They Can be Used Clinically

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In the beginning there were ESRD patients. There were nephrologists. There were transplant surgeons. Eventually there were transplants. And, almost immediately there were rejections!

How to deal with predicting and preventing rejections?
<table>
<thead>
<tr>
<th>Type</th>
<th>Time</th>
<th>Mediated by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute</td>
<td>0-48 hrs</td>
<td>Abs</td>
</tr>
<tr>
<td>Accelerated</td>
<td>5-7 days</td>
<td>Abs/cells</td>
</tr>
<tr>
<td>Acute</td>
<td>Early/delayed</td>
<td>Cells/Abs</td>
</tr>
<tr>
<td>Chronic</td>
<td>&gt;60 days</td>
<td>Abs/cells/?</td>
</tr>
</tbody>
</table>
In 1965 Terasaki reported on a brother to sister transplant. The recipient experienced an immediate rejection of the transplanted renal allograft.

Testing of the pretransplant sera revealed the presence of cytotoxic antibodies vs the donor PBL.

This was the first report of the possible association between pre-graft donor reactive lymphocytotoxic antibodies and immediate graft rejection.

<table>
<thead>
<tr>
<th></th>
<th>Rejection</th>
<th>No Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Crossmatch</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Negative Crossmatch</td>
<td>8</td>
<td>187</td>
</tr>
</tbody>
</table>

P < 0.001

*“the ethics of transplanting kidneys without prior knowledge of the results of the lymphocyte crossmatch test...can reasonably be expected to be questioned.”*
How it All Started…

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Are antibodies present?

Are all antibodies bad?

Therefore, a positive crossmatch became a strict contraindication to transplant!

And so, “on-call HLA lab testing” was born!

The introduction of crossmatching led to a significant decrease in immediate graft rejections.
The purpose of the crossmatch is to detect clinically relevant recipient anti-donor antibodies (HLA, non-HLA) to prevent hyperacute and accelerated rejection.
Evolution of Ab/XM Testing

- CDC
- ELISA
- Flow/Luminex multi-Ag beads
- Virtual XM
- AHG-CDC
- FC Lymphocytes
- Flow/Luminex/ELISA single-Ag and Donor-specific
Determination of Ig Reactivity

NIH-CDC
AHG-CDC
Flow Cytometry

All membrane dependent assays
Complement-dependent Cytotoxicity NIH Assay

Target Cell + IgG + serum → cell surface binding

Target Cell + vital dye → positive reactivity

Target Cell + IgG + IgG → negative reactivity
A negative NIH-CDC means
No recip anti-donor Abs
Too few Abs to cause target cell lysis.
Anti-human Globulin (Enhancement) Assay

- Target Cell + serum + IgG → cell surface binding
- Target Cell + vital dye → No Ab - Few Ab?
- Target Cell + goat anti-human IgG + vital dye →
Flow Cytometry Assay

NIH - CDC Negative
AHG – CDC Negative

Now measuring binding of IgG (absent C’)
Ig Detection by Differing Methodologies

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC</td>
<td>102</td>
<td>162</td>
</tr>
<tr>
<td>AHG-CDC</td>
<td>116 (+13%)</td>
<td>148</td>
</tr>
<tr>
<td>FLOW</td>
<td>139 (+10%)</td>
<td>125</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crossmatch</th>
<th>Sensitivities</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH-CDC</td>
<td>Least</td>
</tr>
<tr>
<td>AHG-CDC</td>
<td>Moderate</td>
</tr>
<tr>
<td>FCXM</td>
<td>Most</td>
</tr>
</tbody>
</table>

Need to pair clinically relevant antibody detection method with most sensitive, clinically relevant crossmatch.
<table>
<thead>
<tr>
<th>POD</th>
<th>NIH</th>
<th>AHG</th>
<th>FCXM</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30</td>
<td>56%</td>
<td>28%</td>
<td>6%</td>
</tr>
<tr>
<td>31-365</td>
<td>25%</td>
<td>18%</td>
<td>9%</td>
</tr>
<tr>
<td>Total</td>
<td>81%</td>
<td>46%</td>
<td>15%</td>
</tr>
</tbody>
</table>
Rejection Rate and Graft Survival in Kidney Transplantation

Stewart F. *Organ Transplantation*, 1999
Positive crossmatches were historically considered a contraindication to transplant based upon the assumption that the reactive recipient Ab was directed against donor HLA.

However, unless specificity or inhibition studies were performed, we never knew whether reactivity was HLA or non-HLA directed.
Membrane-independent Assays

ELISA-determined IgG HLA Abs vs MHC-I (pooled platelets)
ELISA-determined IgG HLA Abs vs MHC-I/II (PBL cultures)
Flow bead PRA-determined IgG HLA vs I/II (soluble HLA I/II antigens on microbeads measured by cytometry)
Antibody Detection using Purified HLA Antigens

Methods: ELISA, Flow Cytometry, Luminex

Targets:
- Mix-antigens from many cells
- Antigens from one cells – Class I, Class II
- Single antigens
ELISA based HLA antibody analysis

Captured Soluble HLA Molecules → Anti-HLA IgG Antibody in Serum → Anti-Human (Enzyme Conjugate)

4. Add enzyme substrate -will turn color in positive wells -read on ELISA reader
Purpose of FlowPRA

The FlowPRA™ screening detects HLA specific antibodies in serum of pre- and post-transplant organ recipients.
How are FlowPRA™ Beads Made?

EBV Cell Line

Purified HLA Antigens

Distinct bead preparations

Latex Microparticles 2-4 µm in diameter
Flow PRA I and Flow PRA II

Bead #1

Bead #2

Bead #3

Beads #4-30

Flow PRA I = Class I antigens
Flow PRA II = Class I antigens

Pooled beads n=30
FlowPRA™ Test

Alloantibody

FITC anti-IgG

FL1

FL2

Class I Bead

Class II Bead
Possible % PRA Determination by FlowPRA

100%

95%

42%

8%
High Definition Microparticles (Single Antigen)

- Microparticles coated with a single HLA molecule (Class I or II)
Luminex-based determination of donor-HLA-specific Ab following kidney transplantation: Case 1

DONOR HLA A11, A24, B7, B15, DR9, DR15

- DR9, 15, 53, 51
Antibody Specificity

Knowing the PRA is not sufficient.

Knowledge of antibody specificity is critical in order predict crossmatch results – Positive or Negative.
We can now identify the presence of HLA-Abs and their Ag specificities. Can this information help in improving pairing of donors to recipients?
New Paradigm

Use new technologies to define “acceptable” HLA mismatches.

That is, instead of pairing donors and recipients by HLA matching we will look for donors that the recipient has no detectable immune reactivity against.

No HLA Abs!
New Paradigm

The ability to identify the presence of IgG-HLA Abs in patient sera allows us to discriminate clinically relevant from irrelevant crossmatches.

Really?
Considerations

1. Ab specificity:
   HLA: A, B, DR / C, DQ, DP
   Non-HLA: MICA, MICB, vascular endothelium, vimentin

2. Type of Ig: IgG (1, 2, 3, 4), IgM, IgA

3. Complement fixing

4. Ab quantity: Titer, Fluorescence intensity (FI)
   Molecules of equivalent soluble fluorescence (MESF)
Problems with Solid-Phase Assays

Limited source of materials (2 vendors)
Unknown Ag density on beads
Flow cytometry vs. Luminex
Need for consistent cut-offs between labs
Tech variation (intra-lab)
Difficult to interpret data based upon above.
Can you identify UAs and make public policy? !
How can you use the data to predict virtual XM?!
Terminology

Donor-Specific HLA Antigen (DSA)

Surrogate Donor HLA Antigen (SDA)
Virtual XM Assumes

1. Accurate detection of all HLA Class I / II Abs present in recipient.

2. The HLA phenotype of the donor. Identification of ALL unacceptable (avoid) antigens (A-, B-, Cw, DRB1, DQB1, DP, DQA?)
Virtual XM Assumes

3. Correct methodologies to identify and call unacceptable antigens

4. PRA is performed with Equivalent Sensitivity as Final Crossmatch
Identifying the presence of an Ab (HLA, non-HLA, Auto) is a phenotypic or demographic description and in no way does it describe the functional capacity and/or clinical consequence of the Ab.

The crossmatch (AHG, FCXM), does measure Ab function.
8-Year Graft Survival

Fluorescence Intensity of Donor Specific Antigen Beads from Crossmatch (FCXM) Positive Sera (N=60) PRA ≥ 80%

<table>
<thead>
<tr>
<th>FI</th>
<th>n</th>
<th>Accumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 – 2,000</td>
<td>6</td>
<td>10%</td>
</tr>
<tr>
<td>2,001 – 3,000</td>
<td>2</td>
<td>13%</td>
</tr>
<tr>
<td>3,001 – 4,000</td>
<td>12</td>
<td>33%</td>
</tr>
<tr>
<td>4,001 – 5,000</td>
<td>6</td>
<td>43%</td>
</tr>
<tr>
<td>5,001 – 6,000</td>
<td>10</td>
<td>60%</td>
</tr>
<tr>
<td>6,001 – 7,000</td>
<td>2</td>
<td>63%</td>
</tr>
<tr>
<td>7,001 – 8,000</td>
<td>4</td>
<td>70%</td>
</tr>
<tr>
<td>8,001 – 9,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9,001 – 10,000</td>
<td>4</td>
<td>77%</td>
</tr>
<tr>
<td>10,001 – 11,000</td>
<td>6</td>
<td>87%</td>
</tr>
<tr>
<td>11,001 – 12,000</td>
<td>1</td>
<td>88%</td>
</tr>
<tr>
<td>12,001 – 13,000</td>
<td>2</td>
<td>92%</td>
</tr>
<tr>
<td>13,001 – 14,000</td>
<td>1</td>
<td>93%</td>
</tr>
<tr>
<td>14,001 – 15,000</td>
<td>2</td>
<td>97%</td>
</tr>
<tr>
<td>≥19,001 -</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>DSA titer</td>
<td>1:1024</td>
<td>1:32</td>
</tr>
<tr>
<td>SCr</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>FCXM</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Rejection</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Clinical Relevance of the XM
Amount of antibody, as reflected by titer and/or fluorescence intensity, may not correlate with FCXM positivity.
How can we Identify a Clinically Relevant (protective / deleterious) Antibody in Solid-Organ Transplantation

1. By a positive crossmatch

2. During a clinical event.

3. As a donor-specifically reactive Ab?

4. As an Ab vs. HLA, specifically donor HLA?

5. As an Ab vs. non-HLA?
How can we Identify a Clinically Relevant (protective / deleterious) Antibody in Solid-Organ Transplantation

6. As an Ab detected by increasingly sensitive methods (NIH, AHG, ELISA, Flow/Luminex) ?

7. As an IgG (1, 2, 3, 4), IgM, IgA ?

8. Complement fixing Ab ?

9. By Ab quantity: Titer, MFI, MESF ?

10. By Ab avidity vs. donor target antigen ?
Identifying the presence and specificity of an Ab (HLA, non-HLA, Auto) is a phenotypic or demographic description and in no way does it describe the functional capacity and/or clinical consequence of the Ab.
Identifying Clinically Relevant Ab

1. Binding of an Ab in a XM is a functional assay which may reflect the amount of Ab, Ab avidity for the target epitope or other factors.

2. Functional assays to identify clinically relevant Abs may be more informative.

3. The cytotoxic (AHG) and/or the binding (FCXM) crossmatches measure Ab function.
Graft Survival vs. Crossmatch

% Survival

Time Post-transplant

Neg. XM

Pos. XM
Graft Survival vs. Crossmatch

- Neg. XM
- Pos. XM

% Survival vs. Time Post-transplant
The Cell Surface Is a Jungle

HLA

Non-HLA — Non-HLA
“Non-HLA Transplantation Immunity Revealed by Lymphocytotoxic Antibodies”

Death-censored Functional Graft Survival of HLA-identical Sibling Transplants

Clq-Fixing Human Leukocyte Antigen Antibodies Are Specific for Predicting Transplant Glomerulopathy and Late Graft Failure After Kidney Transplantation

Julie M. Yabu,1,5 John P. Higgins,2 Ge Chen,2,3 Flavia Sequeira,2,3 Stephan Busque,4 and Dolly B. Tyan2,3

Methods
C1q Assay Based on the Single Ag Bead Technology

IgG Method

C1q Method

Dolly Tyan (Stanford University)
High Specificity of C1q Assay
Identifying Clinically Relevant Ab

1. During a clinical event or by biopsy.
2. During a stable clinical course.
3. Identifying the presence and specificity of Abs does not characterize their function.
4. Binding of an Ab in a XM is a functional assay which may reflect the amount of Ab, Ab avidity for the target epitope or other factors.
5. Functional assays to identify clinically relevant Abs may be more informative.
Do no harm!

Do not deny a potential recipient a donor organ for the wrong reason!

Be an advocate for your patient!

Do not be judgmental!

Equity (do the XM) vs. Utility (Virtual XM)
Conclusions

A functional Ab assay should be correlative to renal dysfunction, rejection and/or graft loss.

Currently, only the crossmatch (cytotoxic, Flow) fulfills the criteria of a functional Ab assay.

The C1q assay may be a surrogate crossmatch assay that may be a functionally descriptive Ab assay.

Therefore, at the present time it is my belief that the crossmatch assays are the only functional Ab assays.