The First Transplant?

Sts. Cosmas and Damian c. 4th Century (modern-day Turkey/Syria)
Host Defense Systems

**Innate**
- external barriers (skin, mucus membranes)
- secretory components (enzymes, histamine, oxygen radicals, etc.)
- certain leukocytes (phagocytes, NK cells, platelets)
- no increase in strength after exposure

**Adaptive (Acquired)**
- evoked during an immune response
- T & B cells
- Leukocytes (monocytes, neutrophils, mast cells)
- Soluble factors (antibodies, cytokines)
The central component of the adaptive immune response is the binding of peptide to HLA (MHC) and the recognition of the complex by T-cells.
Characteristics of HLA (MHC)

- The human MHC is called HLA
- Region of closely-linked highly polymorphic genes encoded on chromosome 6
- Discovered because of transplantation experiments but plays a role in virtually every type of specific response the immune system can perform.
- “self/non-self discrimination”
HLA Antigen Acquisition

Class I

Class II
T-cells “Born” in Bone Marrow

Pre-T-cell

Thymus

Bone
The Thymus

- Positive selection
- Negative selection

- Cortex
- Medulla
- Interlobular Septum
- Thymic Corpuscle
- Thymic Lobule
- Capsule
In The Thymus

**Cell entry**
- Subcapsular zone
  - Cells proliferate and differentiate
  - CD-3 -
  - CD-4 -
  - CD-8 -

**Positive selection**
- Cortex
  - Pre T-cells must be able to recognize HLA
  - CD-3 +
  - CD-4 +
  - CD-8 +

**Negative selection**
- Corticomedulla
  - Pre T-cells must not be able to recognize HLA/normal peptides
  - CD-3 +
  - CD-4 +
  - CD-8 +

**Exit to periphery**
- Medulla
  - T-cells emerge from thymus
  - CD-3 +
  - CD-4 + or -
  - CD-8 - or +

**T-cells are selected to recognize self HLA with foreign peptide**
Three colors in a rectangle!
Modified Rectangle
Modified Rectangles
High Resolution Typing

[Images of American flags]
### Number of HLA Alleles

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB</th>
<th>DQA</th>
<th>DQB</th>
<th>DPA</th>
<th>DPB</th>
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<td></td>
<td>3830</td>
<td>4647</td>
<td>3382</td>
<td>2252</td>
<td>77</td>
<td>1054</td>
<td>44</td>
<td>740</td>
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</tbody>
</table>

$$3.58 \times 10^{23}$$ combinations

(358 sextillion)
Why do HLA Testing?

• Solid organ Transplantation
  • Kidney and K/P
  • Liver
  • Heart and H/L

• Bone Marrow Transplantation

• Platelet Transfusion (Class I only)

• Disease Association

• Parentage Determination
<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA</th>
<th>R.R.</th>
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<tbody>
<tr>
<td>Ankylosing Spondylitis</td>
<td>B27</td>
<td>87.4</td>
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<tr>
<td>Post-gonococcal arthritis</td>
<td>B27</td>
<td>14.0</td>
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<td>Acute Anterior Uveitis</td>
<td>B27</td>
<td>14.6</td>
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<td>Narcolepsy</td>
<td>DR 2</td>
<td>129.0</td>
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<td>IDDM</td>
<td>DR 3</td>
<td>5.0</td>
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<td>DR 4</td>
<td>6.8</td>
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<td>DR 3 &amp; 4</td>
<td>14.3</td>
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<tr>
<td>21-Hydroxylase Deficiency</td>
<td>B47*</td>
<td>15.0</td>
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<td>Rheumatoid Arthritis</td>
<td>DR 4</td>
<td>5.8</td>
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<tr>
<td>Hemochromatosis</td>
<td>A3</td>
<td>8.2</td>
</tr>
<tr>
<td>Abacavir Hypersensitivity</td>
<td>B*5701</td>
<td>?</td>
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</tbody>
</table>
Responsibilities of the HLA Lab

• Determine degree of mismatch between donor and recipient
  – HLA typing

• Identify clinically relevant anti-donor HLA antibodies
  – crossmatch (determines donor/recipient compatibility)
  – serum screening (ongoing evaluation of patient’s immune status)
HLA Typing Techniques

- Mixed Leukocyte Culture
- Complement-dependent Cytotoxicity (CDC)
- DNA Testing
DNA Structure

Helix Backbone
Carbon  Oxygen  Phosphorus

DNA
ATCG
Molecular Typing Methods

- SSP
- SSO
- SBT
- Real-Time qPCR
Examine the LinkSēq Trays
Well Details

• Provides expected curves and peak windows
• Provides a list of specific alleles interrogated in this well
Other testing done by HLA Lab

• **Crossmatching**
  - Donor cells and patient serum
    - Detects antibodies directed against donor Ag

• **Serum Screen**
  - Panel of typed cells reacted against a patient’s serum
    - Detects antibodies to particular HLA Ags
    - Screens out patients with Ab to a donor’s Ag
PRA

- Panel Reactive Antibody
- Percent Reactive Antibody

- Intended to be an assessment of transplantability.

- 20% PRA means that a recipient should crossmatch positive with 20% of donors

- **DO NOT CONFUSE WITH TITER**
Basis of Antibody Testing

Cells or beads + Patient’s serum

Reaction | No reaction
Goals in HLA Antibody Detection

- Is there an anti-HLA antibody present?
  - Serum screen

- Is the antibody clinically relevant?
  - Specificity

- Identification of specific Abs or safe Ags
Luminex Bead Array
Luminex Bead Array
**Determination of Significance**

<table>
<thead>
<tr>
<th>Serum Reactivity</th>
<th>Antigen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>a</td>
<td>+</td>
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<td>+/+</td>
<td>b</td>
<td>-</td>
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<td>+/-</td>
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<td>A = a + b</td>
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<td>B = c + d</td>
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<td>C = a + c</td>
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<td>D = b + d</td>
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<td>T = Total #</td>
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</table>

**Correlation Coefficient (r)**

\[ r = \frac{a \times d - b \times c}{\sqrt{A \times B \times C \times D}} \]

**Chi square \( (\chi^2) \)**

\[ \chi^2 = r^2 \times T \]

*p value depends on degrees of freedom*  
2 x 2 tables have 1 degree of freedom

<table>
<thead>
<tr>
<th>( \chi^2 )</th>
<th>2.7055</th>
<th>3.8415</th>
<th>6.6349</th>
<th>7.8794</th>
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</thead>
<tbody>
<tr>
<td><em>p</em></td>
<td>0.1</td>
<td>0.05</td>
<td>0.01</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Serum reactions

\[
\begin{array}{ccc}
+ & 14 & 3 \\
- & 1 & 21 \\
\end{array}
\]

\[
r = \frac{a*d - b*c}{\sqrt{(A*B*C*D)}}
\]

\[
r = \frac{14*21 - 3*1}{\sqrt{(17*22*15*24)}}
\]

\[
r = 0.793
\]

\[
\chi^2 = r^2 * T
\]

\[
\chi^2 = 0.793^2 * 39
\]

\[
\chi^2 = 24.52
\]

\[
p < 0.0001
\]
**UNET cPRA Screen**

Enter antigens into UNOS database.

<table>
<thead>
<tr>
<th>Unacceptable Antigens</th>
<th>Check all A unacceptable antigens:</th>
<th>2</th>
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<tbody>
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<td>3</td>
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</tbody>
</table>

Select BW unacceptable antigen:

- 4
- 6
- N/A

Check all CW unacceptable antigens:

- 1
- 2
- 3
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Check DR1/52/53 unacceptable antigens:

- 51
- 52
- 53

Check all DQ unacceptable antigens:

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
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- 12
- 13
PRA then calculated

This patient would be expected to have a positive crossmatch with 60% of the UNOS donors in database.
Crossmatch Techniques

• **Complement-dependent cytotoxicity**
  • (original gold standard; usually augmented)

• **Flow cytometry**
  • (new gold standard)

• **Solid Phase**
  • (ELISA & Luminex; in development)
Why Do The Crossmatch?

• 1969 paper by Terasaki’s group

• Initial reason was to detect high levels of pre-formed antibodies that can cause hyperacute rejection.

• As sensitivity increased, tests may detect low-level antibodies that present varying degrees of risk for graft loss

• Required under CLIA (42 CFR 493.1265)
Quotes

“The ethics of transplanting kidneys without the prior knowledge of the results of the lymphocyte crossmatch test…can reasonably be expected to be questioned”

-Patel and Terasaki. NEJM 280:735, 1969

“The ethics of transplanting patients without the prior knowledge of the antibody status can reasonably be expected to be questioned.”

-Robert Bray, Ph.D., 2002
Basis of Antibody Testing

Cells or beads + Patient’s serum

Reaction

No reaction
Flow Cytometer
Flow cytometry interpretations

**T-cell**

**B-cell**

Interpretation for Donor/Recipient XM
(Fluorescent ratio above negative control)

**T-cell Crossmatches**

- 2.0 or less  **Negative**
- > than 2.0  **Positive**

**B-cell Crossmatches**

- 3.0 or less  **Negative**
- > 3.0 < Wk pos ctrl  **Doubtful +**
- > than Weak Pos Ctrl  **Positive**
### Renal and Renal Combinations

- **XM must be done prospectively**

<table>
<thead>
<tr>
<th></th>
<th>Unsensitized Pt.</th>
<th>Sensitized Pt.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-cell XM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative (1)</td>
<td>OK to proceed</td>
<td>OK to proceed</td>
</tr>
<tr>
<td>positive (2, 4, 6, 8)</td>
<td>contraindication</td>
<td>contraindication</td>
</tr>
<tr>
<td><strong>B-cell XM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative (1)</td>
<td>OK to proceed</td>
<td>OK to proceed</td>
</tr>
<tr>
<td>weak pos. (2, 4)</td>
<td>probably OK</td>
<td>probably not good</td>
</tr>
<tr>
<td>strong pos. (6, 8)</td>
<td>probably not good</td>
<td>contraindication</td>
</tr>
</tbody>
</table>
VXM Defined

• A virtual crossmatch (VXM) is a prediction of compatibility based on the patient’s alloantibody (Ab) status when compared to a specific donor’s histocompatibility antigens.

• In other words….

It’s a guess!

»
Rules for Virtual Crossmatching

• Knowledge of antibodies outside of the “normal” HLA is of great importance.
  – False negatives

• Predicting VXM positivity solely on CREGs is not advisable.
  – False positives

• Labs should continuously update a patient’s unacceptable antigens as conditions warrant.
  – Look for antibodies to DP and DQα when FNs crop up.

• VXM is a tool that can help facilitate transplantation but cannot stand alone.
  – Until all antibodies that can affect transplantation are taken into account in match algorithms.
Impact of HLA Ab in Transplantation

• Pre- and peri-transplant
  – Access to transplantation
  – Short-term survival (hyperacute, accelerated)

• Post-transplant
  – Survival
    » Acute and chronic rejection
  – Marker for rejection response
# Classification of Rejection

<table>
<thead>
<tr>
<th>Type</th>
<th>Time after Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute</td>
<td>within minutes</td>
</tr>
<tr>
<td>Accelerated</td>
<td>within 1 month</td>
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<tr>
<td>Acute*</td>
<td></td>
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<tr>
<td>Early</td>
<td>6 – 10 days</td>
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<tr>
<td>Late</td>
<td>11 days – weeks</td>
</tr>
<tr>
<td>Chronic*</td>
<td>months to years</td>
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</tbody>
</table>

*Antibody-mediated (preformed)

*Cellular- and Antibody-mediated
Responsibilities of the HLA Lab

To Assess Degree of Mismatch

&

To identify Clinically Relevant Anti-Donor HLA Antibodies
HLA is central
WFBMC HLA/Immunogenetics Laboratory
www.wakehealth.edu/HLA

• Director – Michael D. Gautreaux, Ph.D., CHT, D. ABHI

• Supervisor – David F. Kiger, BS, CHT, CHS

• Technologists –
  • C. Elaine Forrest, BA, CHT, CHS
  • Jennie Ward, BS, MT(ASCP), CHT, CHS
  • Sharlie B. Brown, BS, CHT, CHS
  • Tabitha L. Peake, BS, CHT, CHS
  • Kimberly Beane, BS, MT(ASCP), CHT
  • Kelly J. Ingram, BS

• Laboratory Assistant – Joanna Fulcher

• Office Coordinator – Patti Shew