Solid Organ Transplantation

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Solid Organ Transplantation

Blood Transfusions in 1600s

Animal to humans: incompatible

Again in the 1800s: incompatible

Karl Landsteiner – work begun in 1901.

Led to description of ABO, M, N, Rh compatible/incompatible transfusions.

Nobel Prize in Medicine
Carrel, C. Guthrie: vascular anastomosis using a fine continuous suture technique, penetrating all vessel layers, resulted in tissue and organ transplants. For this vascular anastomosis procedure, Carrel won the Nobel Prize in Medicine, 1912.

Holman (1924) demonstrated that a single donor’s skin graft applied to a burn patient rejected more rapidly with the second application.

First human kidney transplanted unsuccessfully in 1933 by Voronoy into the groin of a patient in the Ukraine.
Arguments Re: Cellular vs. Humoral Immunity

Ray Owen: Dizygotic twin cattle sharing same circulation in utero became red cell chimeras and were unable to respond immunologically to one another’s antigens.

Burnet: Neonatal antigen exposure may lead to antigen unresponsiveness (tolerance) whereas after this neonatal time period antigen exposure leads to immune response. Nobel Prize in Medicine, 1960.

Billingham, Brent and Medawar described neonatal tolerance in mice. Won Nobel Prize in Medicine, 1960.
During World War II, Peter Medawar, a zoologist interested in skin grafting and Thomas Gibson, a plastic surgeon, demonstrated that a “second set” of skin grafts from a parent to a burned child was rejected more rapidly than the first set. Gibson concluded that “allografts” were of “no immediate clinical use.” For Medawar it was evidence that allograft rejection was a major, unexplained, immunological phenomenon.

Peter Gorer described genetically determined antigens present in host tissue eliciting immune response and destruction (rejection).
Historical Efforts in Transplantation

George Snell: inbred mice, tumors, immune response, MHC, histocompatibility antigens.

This work led to human MHC, HLA A, B, C, DR, DQ, DP antigens.

Benaceraff, Dausset and Snell – Nobel Prize in Medicine, 1980.

1945 saw the development of dialysis machines – Kolff in Holland and Alwell in Sweden.

December 23, 1954 saw the first successful kidney transplant between monosygotic twins. This validated the surgical technique and that without rejection normal health could be restored. The surgeon, Joseph Murray, won the Nobel Prize in Medicine.
Skin graft

Strain A

Skin graft

Strain B

10-14 days

Graft accepted

Skin graft

Strain B

Graft rejected

Transfer Lymphoid cells

5-7 days

ACELERATED REJECTION
FACTS ABOUT TRANSPLANTATION IN THE UNITED STATES

On October 31, 2008, the OPTN National patient waiting list for organ transplant includes the following:

<table>
<thead>
<tr>
<th>Registrations</th>
<th>Description</th>
<th>Waiting List</th>
</tr>
</thead>
<tbody>
<tr>
<td>82,530</td>
<td>registrations for a kidney transplant.</td>
<td>77,746</td>
</tr>
<tr>
<td>16,646</td>
<td>registrations for a liver transplant.</td>
<td>16,053</td>
</tr>
<tr>
<td>1,631</td>
<td>registrations for a pancreas transplant.</td>
<td>1,609</td>
</tr>
<tr>
<td>187</td>
<td>registrations for a pancreas islet cell.</td>
<td>184</td>
</tr>
<tr>
<td>2,329</td>
<td>registrations for a kidney-pancreas transplant.</td>
<td>2,258</td>
</tr>
<tr>
<td>234</td>
<td>registrations for an intestine transplant.</td>
<td>231</td>
</tr>
<tr>
<td>2,714</td>
<td>registrations for a heart transplant.</td>
<td>2,703</td>
</tr>
<tr>
<td>98</td>
<td>registrations for a heart-lung transplant.</td>
<td>98</td>
</tr>
<tr>
<td>2,140</td>
<td>registrations for a lung transplant.</td>
<td>2,124</td>
</tr>
<tr>
<td>108,509</td>
<td>TOTAL REGISTRATIONS</td>
<td>100,487</td>
</tr>
</tbody>
</table>

During 2007 6,527 patients were removed from the OPTN National patient waiting list for reason of death.**
Transplant Considerations:

- ABO compatibility
- Matching for HLA
- Pre-sensitization
Histocompatibility Systems:

1) ABO – Red blood cells
2) HLA – White blood cells
and most body cells

Histo (tissue) Compatibility
Blood Transfusion Success

Donor: A

Recipient:
- Yes → A
- No → B
- Yes → AB
- No → O
In man, the MHC locus is designated as HLA (Human Leukocyte Antigen).

Human MHC Gene Locus

Human HLA Complex

The HLA locus in humans is found on the short arm of chromosome 6.
Methodologies to Evaluate HLA

Serologic typing: HLA B7 vs B51

2) HLA-DNA (PCR Typing) – use of PCR methodology to increase nucleotide sequences and sequence-specific oligonucleotide probes (SSOP), to identify DNA-genomic subtypes
Sequence-specific Primer (PCR-SSP)

The SSP utilizes DNA primers that are specific for individual or similar groups of Class II alleles.

The primers are used with PCR to amplify relevant genomic DNA.
Sequence-specific Oligonucleotide Probes (PCR-SSOP)

Uses locus-specific or group-specific primers to amplify the desired genomic DNA.

This is followed by application of a labeled oligonucleotide probe that binds to an allele-specific sequence.
Phenotype

A32, A33, B65 (W6), B-, CW5, DR1, DR17,
All positive antigens by tissue typing

Genotype

(A32, B65 (W6), CW8, DR1) (A33, B-, CW5, DR17)
Antigens on same chromosomes

Haplotypetype

A32, B65 (W6), CW8, DR1
Antigens on single chromosome
Possible Haplotype Distributions

<table>
<thead>
<tr>
<th>Father</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A2, B8, DR1)</td>
<td>(A23, B44, DR3)</td>
</tr>
<tr>
<td>(A1, B51, DR4)</td>
<td>(A3, B7, DR5)</td>
</tr>
</tbody>
</table>

**HLA identical siblings**

| (A2, B8, DR1) | (A1, B51, DR4) | (A2, B8, DR1) | (A1, B51, DR4) |

**Haplo-identical siblings**

| (A2, B8, DR1) | (A1, B51, DR4) | (A2, B8, DR1) | (A3, B7, DR5) |

**Totally mismatched siblings**

| (A2, B8, DR1) | (A1, B51, DR4) | (A23, B44, DR3) | (A3, B7, DR5) |
### Significance of HLA-A, -B and -DR Typing for AZA+Pred-treated Cadaveric Renal Transplant Recipients

<table>
<thead>
<tr>
<th>Patient HLA mismatches</th>
<th>One-year Graft Survival</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;2A, B, 0-1 DR)</td>
<td>73% (29/40)</td>
<td>-</td>
</tr>
<tr>
<td>(&lt;2A, B, 2 DR)</td>
<td>44% (7/16)</td>
<td>(&lt;0.02)</td>
</tr>
<tr>
<td>(&gt;2A, B, 0-1 DR)</td>
<td>54% (21/39)</td>
<td>-</td>
</tr>
<tr>
<td>(&gt;2A, B, 2 DR)</td>
<td>38% (6/16)</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>
Effect of HLA-B or -DR-mismatches

Without CsA

With CsA

% Graft Survival

Time (months)

P<0.0001

P<0.0001

0 (n=381)

1 (n=383)

2 (n=1919)

3 (n=1113)

4 (n=378)

0 (n=161)

1 (n=561)

2 (n=693)

3 (n=451)

4 (n=181)
One-year Graft Survival for HLA-identical Sibling, Parental and Cadaveric Donor Transplants

% Survival

Months Post-transplant

1982-1985

1992-1995

2002-2005

1985 1995 2005

HLA-id Sib Donor 641 1,343 2,081

Parent Donor 590 1,983 3,165

Cadaver Donor 6,026 24,449 36,474
Effect of HLA-A –B and –DR Mismatching on Graft Survival
Donor-recipient HLA incompatibility can result in an immune response, rejection and possible graft loss. Immunosuppressants may obviate the impact of HLA-matching for both short and long-term graft outcome.
Key Terms:

**Autograft:** a graft or transplant from one area to another on the same individual.

**Isograft:** a graft or cells from one individual to another who is syngeneic (genetically identical) to the donor.

**Allograft:** graft or transplant from one individual to an MHC-disparate individual of the same species.

**Xenograft:** graft between a donor and a recipient from different species.
Types of solid organ transplants:
  - Kidney
  - Liver
  - Heart
  - Lung
  - Pancreas
  - Intestine

Deceased donors (D-D): formerly cadaveric donors (CAD)

Living donors:
  - Living related donors (LRD)
  - Living unrelated donors (LURD)
Transplant Considerations

- ABO compatibility
- Matching for HLA
- Pre-sensitization
<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>
<tr>
<td></td>
<td>Immune</td>
<td>Trauma</td>
</tr>
<tr>
<td></td>
<td>Non-immune</td>
<td></td>
</tr>
</tbody>
</table>
HLA Ab Sensitization

Pregnancy

Blood transfusions

Failed allograft

Some types of bacterial infections
## HLA Antigen Expression in the Kidney

<table>
<thead>
<tr>
<th>Vasculature</th>
<th>Glomerulus</th>
<th>Tubules</th>
<th>Interstitium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td>Endo</td>
<td>Prox</td>
<td>Endo</td>
</tr>
<tr>
<td>Cap.</td>
<td>Mesan</td>
<td>Dist.</td>
<td>Mesan</td>
</tr>
<tr>
<td>Class I</td>
<td>Epi</td>
<td>Dendritic</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>++ 0/+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>++</td>
<td>0/+ 0/+</td>
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<td>++ 0 0</td>
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<td>0+</td>
<td>++ 0 0</td>
<td>+</td>
<td>++</td>
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<tr>
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<td>++ 0/+ 0</td>
<td>+</td>
<td>+++</td>
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<th>Dist.</th>
<th>Dendritic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0/+</td>
<td>0/+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Class II: 0+  ++  ++  0  0  0/+  0  +++
## Why Pre-transplant Crossmatches are Performed

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

\[ P = 8.18 \times 10^{-29} \]

Patel & Terasaki, *NEJM*; 280:735, 1969
Detection of Antibody to Donor (HLA) Antigens

Antibody screen

Crossmatch
Screen sera for reactivity vs target cells by cytotoxicity/fluorescence readouts.

Since a patient’s Ab response could fluctuate, serum evaluations must be done at several time points.

Use the most informative sera when performing the recipient vs donor crossmatch (historically most reactive, current and pretransplant sera).
Variation in Lymphocytotoxic Abs

% Reactions with Random Donor Lymphocytes

Months
PRA
Panel Reactive Antibody
Percent Reactive Antibody
Serum Screening Procedure

Tray loaded with individual patient sera

Formalin → Eosin

Unrelated individual

Peripheral blood lymphocytes + C'

Dead cells stained with Eosin
Ab in that patients serum

Viable cell, no Ab
Panel-reactive Antibody (PRA)

Peak : Current
(Historical past) (Recent)

90 : 40
Determination of % PRA

NIH-CDC

AHG-CDC

Flow cytometry

Membrane-dependent Assays
Complement-dependent Cytotoxicity NIH Assay

Target Cell + IgG + serum → cell surface binding → Target Cell

Target Cell + vital dye → positive reactivity

Target Cell + IgG + IgG → negative reactivity
Flow Cytometry Assay
NIH - CDC Negative
AHG – CDC Negative
Now measuring binding of IgG (absent C')
<table>
<thead>
<tr>
<th>Type</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</td>
<td>148</td>
</tr>
<tr>
<td>Flow</td>
<td>139</td>
<td>125</td>
</tr>
</tbody>
</table>
The Cell Surface Is a Jungle

HLA
The Cell Surface Is a Jungle
Membrane-dependent Assays

NIH-CDC
AHG-CDC
Flow cytometry

Detection of membrane receptors may not be related to HLA!
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)
Flow PRA I and Flow PRA II

Bead #1

Flow PRA I = Class I antigens

Bead #2

Flow PRA II = Class I antigens

Bead #3

Pooled beads n=30

Beads #4-30
Correlation of Pre-transplant Abs Detected by Flow PRA with Biopsy-documented Cardiac Rejection


Rejection-free Survival

Cases
- None 65
- Yes 29

P<0.001

Months Post-transplant

Crossmatch

Recipient serum + Donor cells = RXN

**Cells alive** = Negative

**Cells dead** = Positive
The purpose of the crossmatch is to detect clinically relevant IgG anti-donor antibodies to prevent hyperacute, accelerated or chronic rejection.
Detection of Donor-Reactive Antibodies

NIH-CDC

AHG-CDC

Flow cytometry
## Cadaveric Renal Allograft Survival Among 1º CsA-Pred Recipients at 12 months

<table>
<thead>
<tr>
<th></th>
<th>NIH</th>
<th>AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neg.</strong></td>
<td><strong>n=166, 81% (134/166)</strong></td>
<td><strong>n=151, 82% (124/151)</strong></td>
</tr>
<tr>
<td><strong>Pos.</strong></td>
<td><strong>n=15, 67% (10/15)</strong></td>
<td><strong>P&lt;0.01</strong></td>
</tr>
</tbody>
</table>

Cadaveric Renal Allograft Survival Among 1° CsA-Pred Recipients at 12 months

<table>
<thead>
<tr>
<th></th>
<th>AHG</th>
<th>DTE-AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos.</td>
<td>n=15</td>
<td>n=12</td>
</tr>
<tr>
<td></td>
<td>67%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td>(10/15)</td>
<td>(10/12)</td>
</tr>
</tbody>
</table>

\[ P < 0.01 \]

<table>
<thead>
<tr>
<th></th>
<th>T-FCXM</th>
<th>T-FCXM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos. n=148</td>
<td>75%</td>
<td>Neg. n=693</td>
</tr>
<tr>
<td>75%</td>
<td></td>
<td>82%</td>
</tr>
</tbody>
</table>

\[ P < 0.01 \]

Now we can identify HLA Abs.

Also we can identify the HLA Ab specificity that is anti-HLA 1 or HLA B5.

Therefore, we can drop the term PRA and refer to the specific HLA Ab specificity in patient sera and it’s strength.
Immunosuppressive Drugs to Prevent Allograft Rejection

At the present time there is no clinical protocol to induce tolerance to allografts. Therefore, all patients require daily treatment (for a life-time) with immunosuppressive agents to inhibit rejection. All the immunosuppressive agents used in clinical practice have drawbacks relating either to toxicity and side effects or to the failure to provide sufficient immunosuppression. On one hand, excessive immunosuppression can lead to development of opportunistic infections and neoplasia. On the other hand, inadequate immunosuppression allows the recipient to mount the immune response, causing allograft rejection.
**Immunosuppressives:**

- Azathioprine (*Imuran*)
- Steroids
- Cyclosporine (*Neoral*)
- Tacrolimus (*Prograf*)
- Sirolimus (*Rapamune*)
- Mycophenolate mofetil (*Cellcept*)

**Anti-lymphocyte preparations:**

- Thymoglobulin (anti-T, B, NK, etc.)
- Anti-CD3 (*OKT3*), anti-CD20 (*Rituximab*)
- Anti-CD54 (*Campath*)
- Bortezimib (*Velcade*), a B Cell proteasome inhibitor
To Transplant or Not to Transplant?

<table>
<thead>
<tr>
<th>HLA Ab</th>
<th>FCXM</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>(-)</td>
<td>Tx</td>
</tr>
<tr>
<td>(+)</td>
<td>(-)</td>
<td>Tx (?)</td>
</tr>
<tr>
<td>(-)</td>
<td>(+)</td>
<td>?</td>
</tr>
<tr>
<td>(+)</td>
<td>(+)</td>
<td>high-risk for rejection and graft loss</td>
</tr>
</tbody>
</table>