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
In the year 1491, Pope Innocent VIII, had an apoplectic stroke and fell into a kind of somnolence, which was sometimes so profound that the whole court believed him to be dead. All means to awaken the Pope’s exhausted vitality were tried in vain.

Finally, a physician proposed to use a new instrument to exchange the blood of a young person with that of the Pope. Hitherto this experiment had only been done on animals.

Accordingly, the blood of the decrepit old pontiff was passed into the veins of a youth, whose blood was transferred into the veins of the old man. The experiment was tried three times at the cost of the lives of three boys (and 36 units of blood). The Pope also died and the physician quickly disappeared!

- Vallari
Historical Efforts in Transplantation

Chinese: Second century B.C.

Cosmas and Damian: 285 - 305 A.D.

Casparo Tagliacozzi: 1547 - 1599

A. 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.
Cosmas and Damian
Gasparo Tagliacozzi
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.

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

First human kidney transplanted unsuccessfully in 1933 by Voronoy into the groin of a patient in the Ukraine.

During WW 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.
Solid Organ Transplantation

During World War II Medawar re-examined this “second-set” phenomenon and established that rejection of foreign skin grafts followed all the rules of immune specificity.

Billingham, Brent and Medawar described neonatal tolerance in mice. Won Nobel Prize in Medicine, 1960.

Peter Gorer: described genetically determined antigens present in host tissue elicited immune response and destruction (rejection).

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

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

Benacerraff, Dausset and Snell – Nobel Prize in Medicine, 1980.
1945 saw the development of dialysis machines – Kolff in Holland and Alwell in Sweden.

1945, Hufnagel et al joined the vessel of a cadaveric kidney to the brachial vessels of a comatose workman suffering from acute renal failure from septicemia – the patient recovered.

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.
# 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>Patients Waiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>82,530 kidney transplant.</td>
<td>77,746 kidney transplant.</td>
</tr>
<tr>
<td>16,646 liver transplant.</td>
<td>16,053 liver transplant.</td>
</tr>
<tr>
<td>1,631 pancreas transplant.</td>
<td>1,609 pancreas transplant.</td>
</tr>
<tr>
<td>187 pancreas islet cell.</td>
<td>184 pancreas islet cell.</td>
</tr>
<tr>
<td>234 intestine transplant.</td>
<td>231 intestine transplant.</td>
</tr>
<tr>
<td>2,714 heart transplant.</td>
<td>2,703 heart transplant.</td>
</tr>
<tr>
<td>98 heart-lung transplant.</td>
<td>98 heart-lung transplant.</td>
</tr>
<tr>
<td>2,140 lung transplant.</td>
<td>2,124 lung transplant.</td>
</tr>
<tr>
<td><strong>TOTAL REGISTRATIONS</strong></td>
<td><strong>TOTAL PATIENTS</strong></td>
</tr>
<tr>
<td>108,509</td>
<td>100,487</td>
</tr>
</tbody>
</table>

Numbers of Transplants Performed, 2007**

- 16,624 kidney (no pancreas) transplants (6,037 living donors)
- 6,493 liver transplants.
- 469 pancreas (no kidney) transplants.
- 862 kidney-pancreas transplant.
- 198 intestine transplants.
- 2,210 heart transplants.
- 30 heart-lung transplants.
- 1,469 lung transplants.
- 28,355 TOTAL

Number of Donors Recovered, 2007**

- 8,091 Deceased
- 6,308 Living
- 14,399 TOTAL

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)
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

**Haplotype**

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>(A1, B51, DR4) (A3, B7, DR5)</td>
</tr>
<tr>
<td>(A1, B51, DR4)</td>
<td></td>
</tr>
</tbody>
</table>

#### HLA identical siblings

<table>
<thead>
<tr>
<th>(A2, B8, DR1)</th>
<th>(A1, B51, DR4) (A2, B8, DR1)</th>
</tr>
</thead>
</table>

#### Haplo-identical siblings

<table>
<thead>
<tr>
<th>(A2, B8, DR1)</th>
<th>(A1, B51, DR4) (A2, B8, DR1)</th>
<th>(A3, B7, DR5)</th>
</tr>
</thead>
</table>

#### Totally mismatched siblings

<table>
<thead>
<tr>
<th>(A2, B8, DR1)</th>
<th>(A1, B51, DR4)</th>
<th>(A23, B44, DR3)</th>
<th>(A3, B7, DR5)</th>
</tr>
</thead>
</table>
% Graft Survival

Years

% Graft Survival

HLA Identical Siblings (n=1424)

Parent (n=1981)

Cadaver (n=11861)
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

P<0.0001  P<0.0001

Time (months)
One-year Graft Survival for HLA-identical Sibling, Parental and Cadaveric Donor Transplants

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>Mesan</td>
<td>Epi</td>
</tr>
<tr>
<td>Class I</td>
<td>++</td>
<td>++</td>
<td>0/+</td>
</tr>
<tr>
<td>Class II</td>
<td>0+</td>
<td>++</td>
<td>0</td>
</tr>
</tbody>
</table>
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

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PRA
Panel Reactive Antibody
Percent Reactive Antibody
Serum Screening Procedure

Tray loaded with individual patient sera + Unrelated individual peripheral blood lymphocytes + C'

Formalin → Eosin

- 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
- Vital dye
- Cell surface binding
- Positive reactivity
- Negative reactivity
Flow Cytometry Assay

NIH - CDC
Negative

AHG – CDC
Negative

Now measuring binding of IgG (absent C’)

L

L
## Abs by Different Methodologies

<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

Bead #2

Bead #3

Beads #4-30

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

Pooled beads
n=30
Correlation of Pre-transplant Abs Detected by Flow PRA with Biopsy-documented Cardiac Rejection

Tambur et al, Transplantation; 70:1055, 2000
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 1° CsA-Pred Recipients at 12 months**

<table>
<thead>
<tr>
<th>NIH</th>
<th>AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>n=166</td>
<td>n=151</td>
</tr>
<tr>
<td>81%</td>
<td>82%</td>
</tr>
<tr>
<td>(134/166)</td>
<td>(124/151)</td>
</tr>
<tr>
<td></td>
<td>67%</td>
</tr>
<tr>
<td></td>
<td>(10/15)</td>
</tr>
</tbody>
</table>

\[ P < 0.01 \]

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

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

$p<0.01$

<table>
<thead>
<tr>
<th>T-FCXM</th>
<th>T-FCXM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos.</td>
<td>Neg.</td>
</tr>
<tr>
<td>n=148</td>
<td>n=693</td>
</tr>
<tr>
<td>75%</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 its 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*)
Azathioprine (AZA):
AZA (Burroughs-Wellcome, NC) is an S-imidazole derivative of 6-mercaptopurine which inhibits de novo DNA synthesis. Although AZA inhibits the primary immune responses, it has little effect upon the secondary responses. AZA was the first immunosuppressive drug successfully used in organ transplantation. Today AZA is only used clinically in combination with other immunosuppressive drugs.

Corticosteroids:
Corticosteroids are used in patients treated with AZA, CsA or TAC as a mandatory addition to produce an effective immunosuppression in transplant patients. Corticosteroids used in clinical transplantation allow physicians to lower the doses of other immunosuppressive drugs.
Cyclosporine (CsA):
CsA (Sandimmune; Novartis Pharmaceuticals; Switzerland) a cyclic polypeptide produced by *Tolypocladium inflatum* fungi is very effective immunosuppressant for preventing allograft rejection. CsA has a selective (but reversible) inhibitory effect on T helper lymphocytes by blocking the production of IL-2, IFN-γ, IL-4 and other cytokines. In particular, in the cytoplasm CsA binds to immunophilin (CyPA) and CsA-CyPA complex blocks the function of enzyme calcineurin (CaN). In effect, CaN fails to dephosphorylate the cytoplasmic compound of the nuclear factor of activated T cell (NF-ATc), thereby preventing IL-2 (or other cytokine) gene transcription.
Tacrolimus (TAC- FK506):

TAC (Fujisawa Pharmaceuticals, Japan), a macrolide, is produced by *Streptomyces tsukubaensis*. TAC forms a complex with FK binding protein (FK-BP), and the TAC/FK-BP complex blocks calcinuerin function preventing IL-2 (and other cytokine) gene transcription. Tacrolimus is presently used for kidney, heart, liver, lungs and pancreas transplantation.
Sirolimus (SRL):

SRL (Rapamycin; Wyeth-Ayerst, Princeton, NJ) is a macrolide antibiotic produced by *Streptomyces hygroscopicus*. SRL molecule binds to the FK-BP and to the mammalian target of rapamycin (mTOR). In contrast to CsA and FK506, SRL does not block cytokine production but instead inhibits cytokine signal transduction, for example IL-2 cytokine/IL-2 receptor. Interestingly, SRL is particularly effective when used in combination with CsA or TAC by producing a potent synergistic immunosuppressive interaction. At present, the SRL/TAC as well as SRL/TAC combinations are used in clinical therapy.
Biological Products Used for Immunosuppression:

In addition to drugs, polyclonal sera are prepared by immunization of animals with human lymphocytes to produce anti-lymphocyte serum (ALS). ALS is used to treat the incidence of rejection or as induction therapy shortly after transplantation (Thymoglobulin). Furthermore, murine monoclonal antibodies (MAb) directed to CD3 molecules (Orthoclone, OKT3) are licensed for use in clinical organ transplantation. The OKT3 MAb reacts with one of the CD3 molecules expressed on T cells and blocks their function. Treatment with OKT3 MAb inhibits allograft rejection, by lowering the number of circulating T lymphocytes. However, both these antibodies may induce potent immune responses, thereby limiting the duration of treatments.
## To Transplant or Not to Transplant?

<table>
<thead>
<tr>
<th>HLA Ab</th>
<th>FCXM</th>
<th>Tx</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</td>
</tr>
</tbody>
</table>

for rejection and graft loss