Immunology of Transplantation
Introduction - 1

- A process of taking cells, tissues, or organs (a graft), from one individual and placing them into a (usually) different individual to replace diseased ones.

- Transplanted tissues are either autografts, syngrafts, allografts, or xenografts.

- The most commonly transplanted in clinical practice are allografts.
• Genetic disparity between the donor and recipient represents transplantation barriers.

• A major limitation is the immune response of the recipient to the donor tissue.

• First appreciated when attempts to replace damaged skin on burn patients were uniformly unsuccessful.

• Such responses are referred to as rejection.
Graft Rejection - 1

- Classical immunologic principles cannot be applied to understand the field of transplantation.

- Allogeneic responses differ from other immune responses in at least two fundamental aspects:
  - First, they exhibit extraordinary strength.
  - Second, they can be stimulated by two different sets of antigen-presenting cells; namely those of the donor and those of the recipient.
Graft Rejection - 2

• **Direct Recognition of Alloantigens**
  - T-cells can recognize allogeneic MHC antigens directly without the usual requirement of processing and presentation by APCs.

• **Indirect Recognition of Alloantigens**
  - The recipient APCs process and present shed donor class I and class II MHC antigens. As a consequence, CD4+, CD8+, B cells and other effector mechanisms are activated.
Direct allore cognition

Allogeneic MHC

Allogeneic antigen-presenting cell in graft

Alloreactive T cell

T cell recognizes unprocessed allogeneic MHC molecule on graft APC
Indirect alloantigen presentation

Uptake and processing of allogeneic MHC molecules by recipient APC

Presentation of processed peptide of allogeneic MHC molecule bound to self MHC molecule
**Direct recognition**

Host T cell recognizes combination of donor MHC and foreign peptide.

**Indirect recognition**

Host T cell recognizes combination of host MHC and foreign peptide. Donor-derived peptide presented by recipient APC. Alloantigen.
Graft Rejection - 3

- **Direct Recognition**
  - similarity of determinants expressed by allo MHC antigens with those created by the presentation of peptides by self MHC molecules.
  
  - A cross reaction of a normal TCR which was selected to recognize a self MHC molecules plus a foreign peptide, with an allogeneic MHC molecule plus peptide (Anti-TCR antibody inhibit such recognition).
Simple model of T cell-mediated rejection
Complex model of T cell sensitization pathways
Complex mode of T-cell effector pathways
• Following activation, T cells, B cells and monocytes enter the graft.

• The highly polymorphic MHC molecules almost always trigger a response against the grafted organ.

• Matching at MHC is possible only between relatives.

• Minor Histocompatibility Antigens (> 30) cause rejection at different rates.
Mechanisms of Graft Rejection

At least four distinct mechanisms have been identified so far: *hyperacute, accelerated, acute, and chronic.*

However, it is increasingly possible to characterize these mechanisms according to the cell types and processes involved and, in some cases, they may occur at uncharacteristic times.
Graft Rejection - 6

- Rejection caused by preformed Antibodies (Hyperacute) - 1
  - Rejection of a vascularized organ within minutes to hours after transplantation.
  - Transplanted kidneys turn blue and mottled shortly after vascularization is established.
  - Extensive vascular thrombosis and hemorrhage with little evidence of a mononuclear cell infiltrate.
Graft Rejection - 7

• Hyperacute Rejection - 2

• Several important components:
  - Donor endothelial MHC antigens or carbohydrate determinants.
  - Preformed antibodies that can bind these antigens.
  - The complement and coagulation cascades which are activated.
  - Complement regulatory proteins that can modify complement activation, and anticoagulants that can modify the coagulation pathway.
• **Hyperacute Rejection - 3**
  - The target is the donor vascular endothelium.
  - The crucial event is the formation of the membrane attack complex (MAC).
  - Complement activation is controlled by several regulatory molecules [sCr1, DAF(CD55), MCP(CD46), and CD59] which act at different stages along the cascade.
  - Initial stimulus for activation must be strong enough to overcome these down regulating molecules.
Graft Rejection - 9

• Hyperacute Rejection - 4

• Preformed anti MHC antibodies almost always accomplish activation, whereas the lower affinity blood group antibodies lead to hyperacute rejection in only about 25% of cases.

• Hyperacute rejection is such an important feature in xenografting because complement regulatory proteins produced by the donor vascular endothelium of one species do not always function effectively with complement molecules derived from a different species (homologous restriction).
Schematic Representation of Hyperacute Rejection

Antigen

Endothelium

Antibody

Complement Activation

Complement Regulatory Proteins

DAF
MCP
CD59
sCr1
• **Hyperacute Rejection - 5**

• **Type 1 Endothelial Activation:**
  - Due to the effect of MAC on the donor vascular endothelium, even before cell lysis. Manifestations of this activation are:
    a) cell retraction, leading to gaps between endothelial cells,
    b) loss of antithrombotic molecules from the endothelium.
  - Thus, type 1 endothelial activation is responsible for the two principal pathologic findings in hyperacute rejection:
    a) extravascular hemorrhage and edema
    b) intravascular thrombosis
Figure 17-7

1. Preexisting host antibodies are carried to kidney graft

2. Antibodies bind to antigens of renal capillaries and activate complement (C~)

3. Complement split products attract neutrophils, which release lytic enzymes

4. Neutrophil lytic enzymes destroy endothelial cells; platelets adhere to injured tissue, causing vascular blockage
• Hyperacute Rejection – 6

• **No treatment** can stop the process of hyperacute rejection once it started, thus it is essential to avoid the circumstances that initiate it by avoiding transplantation in the face of preformed antibodies (cross match and blood group compatibility).

• Not all organs and tissues are equally susceptible to hyperacute rejection.
Hyperacute Rejection - 7

Although hyperacute rejection is a dramatic and powerful mechanism of graft rejection, it is rarely encountered in clinical practice.

The underlying of its causes, and the use of standard immunologic assays to detect preformed antibodies, has largely eliminated its occurrence.

This is one of the best examples where an understanding of immunology has had an important impact on clinical transplantation.
Graft Rejection - 13

- Early Rejection caused by induced Antibodies (Accelerated Rejection)

- Mediated by antibodies induced within 5 days of transplantation

- Fibrinoid necrosis of donor arterioles with intravascular thrombosis.

- Rare because it requires that an antibody response occurs before the T-cell response.

- Transplanted organs can survive in the face of circulating antibodies that can bind endothelial antigens (accommodation).
Graft Rejection - 14

• Rejection Caused by T-cells (Acute Rejection) - 1

• first-set rejection (11-15 days)

• second-set rejection (6-8 days)

• Most rejections are of this type with decreasing frequency after the first three months.

• Strategies have been developed leading to improvement in graft survival (>80% for one year).
Graft Rejection - 15

• Rejection Caused by T-cells (Acute Rejection) – 2

• Allogeneic MHC antigens stimulate T cell-responses, especially by direct recognition of these antigens. Indirect recognition is also involved, but in decreasing importance (CD4 direct → CD4 indirect → CD8 direct).

• Relative importance depends on type of graft, antigenic disparity, time of transplantation and the previous history of recipient.

• Effector mechanisms include DTH, cytotoxic T cells, cytokines, toxic molecules (nitric oxide) and NK cells.
Graft Rejection - 16

• Rejection Caused by T-cells (Acute Rejection) - 3

• Responses to minor histocompatibility antigens are much less potent than responses to MHC differences because the frequency of responding T cells is much lower.

• CD8+ T cells respond to minor H antigens implying that these antigens are peptides bound to self MHC class I molecules. However, peptides bound to class II molecules can also participate in the response.
Graft Rejection - 17

• Rejection Caused by T-cells (Acute Rejection) - 4

• Virtually any protein made by a cell has the potential to produce peptides that can be recognized as minor H antigens.

• As all cells in a graft express minor H antigens, the entire graft may be destroyed.

• Even with MHC matching, polymorphism at any protein may elicit potent T cell responses.

• It is no wonder that successful transplantation requires the use of potent immunosuppressive drugs.
Graft Rejection - 18

- Chronic Rejection (B and/or T-cell mediated) - 1

- Even when 1 year graft survival has been achieved, the loss of transplanted organs continues to occur at a rate of about 3-5% per year and a significant portion of this loss appears to be due to immunologic mechanisms.

- The term “chronic rejection” has been used to describe this late process of graft destruction.

- Chronic rejection has emerged as one of the most important problems in clinical practice.
Graft Rejection - 19

- Chronic Rejection (B and/or T-cell mediated) - 2

- The half-life for renal transplants that have survived for 1 year has not changed significantly over the last 30 years (about 50% of transplants are still functioning 10 years later).

- Pathologic manifestations vary but always involve narrowing of the vascular bed.

- Important observations made are; the presence of anti-donor antibody, refractoriness to increases in immunosuppression, and a high correlation between the onset of chronic rejection and history of early acute rejection episodes.
Graft Rejection - 19

• Chronic rejection
  • Caused by both antibody and cell-mediated immunity
  • May occur **months to years** down the road in allograft transplants after normal function has been assumed
  • Important to point out rate, extent, and underlying mechanisms of rejection that vary depending on tissue and site
  • The recipient’s circulation, lymphatic drainage, expression of MHC antigens and other factors determine the rejection rate
  • Inflammation, smooth muscle proliferation, fibrosis
  • Tissue ischemia
Figure 16-6 Histopathology of different forms of graft rejection.

A. Hyperacute rejection of a kidney allograft with endothelial damage, platelet and thrombin thrombi, and early neutrophil infiltration in a glomerulus.

B. Acute rejection of a kidney with inflammatory cells in the interstitium and between epithelial cells of the tubules.

C. Acute rejection of a kidney allograft with destructive inflammatory reaction destroying the endothelial layer of an artery.

D. Chronic rejection in a kidney allograft with graft arteriosclerosis. The vascular lumen is replaced by an accumulation of smooth muscle cells and connective tissue in the vessel intima. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston.)
1. Macrophage – T cell mediated
2. Concentric medial hyperplasia
3. Chronic DTH reaction
Manipulations to Prevent Graft Rejection - 1

- Donor-Recipient Matching
- MHC matching
  - Improves the success rate but does not prevent rejection.

  - HLA typing is imprecise owing to the polymorphism and complexity of the human MHC.

  - Grafts between HLA identical siblings are invariably rejected, albeit more slowly, unless donor and recipient are identical twins (minor H antigens).
Manipulations to Prevent Graft Rejection - 2

• Donor-Recipient Matching
• MHC matching
• The current success of clinical transplantation of solid organs is more the result of advances in immunosuppressive therapy than of improved tissue matching.

• The limited supply of organs coupled with the urgency of identifying a recipient once a donor becomes available, means that accurate matching of tissue types is achieved only rarely
Manipulations to Prevent Graft Rejection - 3

- Donor-Recipient Matching
- MHC matching

- Matching only HLA-A, HLA-B, and HLA-DR is important for predicting outcome (0-6 antigen-matching).

- Typing with antibodies for class II alleles is especially imprecise (secondary MLR to detect splits).

- Recently, PCR has been used to permit more complete typing of class II loci and has replaced both serology and secondary MLR.
Manipulations to Prevent Graft Rejection - 4

• Donor-Recipient Matching
• Tests to be Done
  - ABO typing
  - HLA (class 1 and class II) matching
  - MLC
  - Cross matching
Tissue typing

• **Microcytotoxicity assay**
  – Known antibody to WBCs of donor / recipient
  – Complement mediated lysis if Ab present on cell surface

• **Mixed lymphocyte culture (MLC)**
  – Irradiated donor lymphocytes (stimulants)
  – Incubated with recipient lymphocytes
  – $^3$H Thymidin incorporation measured

• **Flow cytometry cross typing**

• **DNA analysis**
  – Genomic typing (very precise, many subtypes)
• Transplantation almost invariably results in some form of rejection.

• Strategies used to avoid or delay rejection are general immunosuppression and minimizing the strength of the specific allogeneic reaction.

• Approaches for inducing donor-specific tolerance are also nearing clinical trials.
Prevention and Treatment of Rejection-2

• **Immunosuppressive Drugs**

• **Inhibit or lyse T Lymphocytes:**
  - **Cyclosporine A**: blocks IL-2 dependent growth and differentiation of T cells.
  - **Tacrolimus** (FK 506): inhibits T cell activation.
  - **Rapamycin**: inhibits T cell proliferation.
Prevention and Treatment of Rejection

- **Metabolic Toxins** that kill proliferating T cells
  - Inhibit maturation of lymphocytes and kill proliferating mature T cells that have been stimulated by alloantigens.

- **Mycophenolate mofetil** is the newest of these agents that is routinely used with Cyclosporine-A.
Prevention and Treatment of Rejection

- **Antibodies reactive with T cell surface structures**
  - Anti CD3, anti-CD25 (α subunit of IL-2 receptor), anti-CD4, anti-CD8, and anti-ICAM

- **Anti Inflammatory agents**
  - Corticosteroids: inhibit synthesis and secretion of cytokines including TNF and IL-1 by mononuclear phagocytes.

- **Tolerance Induction**
  - Blood transfusion, Soluble CTLA-4, Anti-CD40 ligand, Anti-IL-2 receptor, and MHC donor peptide.
T cells that recognize graft antigens lack costimulation and become anergic.

Graft survives.
T-regulatory cell function

**Benefits:**
- T-cell homeostasis
- Prevents autoimmune disease
- Tolerance after transplantation
- Prevents GVHD
- Prevents allergy
- Prevents hypersensitivity

**Detrimental effects:**
- Down-regulation of tumour immunity
- Down-regulation of immunity to infection

*Nature Reviews | Immunology*
If T reg cells can be induced to recognize the indirect antigen presentation, they exert a powerful suppressive effect on both indirect and direct CD4 and CD8 cell activity through the secretion of IL-10 and TGF-β.
Transplant tissue

CD40

CD40L

Anti-CD3 mAb

Anti-CD40L

TCR

B7

B7

CTLA-4Ig

CTLA-4

CTLA-4Ig

Anti-CD25 mAb

IL-2Rα

(CD25)

Costimulation

T_H cell

PLCγ

Cyclosporin A

Calcineurin

JAK3

JAK3 inhibitor

FK506

Nucleotide synthesis

Azathioprine

Cyclophosphamide

Mycophenolate mofetil

Methotrexate

Cell cycle

Rapamycin

Figure 17-10
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Clinical Transplantation - 1

• Historical Background

• The Hindu surgeon Sushrutu (700 BC) used forehead flap to repair an amputated nose.

• Italian surgeons (15th century) began to practice rhinoplasty by flaps and extended the donor site to the patient’s arm.

• Skin grafting became an accepted practice in the late 1800’s.

• The results of these efforts led to a period of confusion in transplantation.
Surgeons embarked on all sorts of transplants (Dr. Serge Voronoff procedure).

Transplantation of internal organs after the development of techniques of vascular anastomosis by the mid of the 20th century.

The first successful renal transplant was performed in 1954 in Boston using the kidney of an identical twin.

Common transplants include; skin, cornea, kidney, heart, lung or heart/lung, liver, bone marrow, small bowel, pancreas or islets, and brain cells.
Figure 17-11
Kuby IMMUNOLOGY, Sixth Edition
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**Cornea**
- From cadaver
- Immunosuppression not required
- 47,000 transplants in 2005

**Skin**
- Mostly autologous (burn victims)
- Temporary grafts of nonviable tissue
- Allogeneic grafts rare, require immunosuppression

**Lung**
- From brain-dead donor
- Procedure recently developed; little data available
- 1408 transplants in 2005
- Often heart/lung transplant (33 in 2005)

**Blood**
- Transfused from living donor
- ABO and Rh matching required
- Complications extremely rare
- An estimated 14 million units used each year

**Pancreas**
- From cadaver
- Islet cells from organ sufficient
- 540 transplants in 2005
- Increasingly, pancreas/kidney transplant for advanced diabetes (903 in 2005)

**Heart**
- From brain-dead donor
- HLA matching useful but often impossible
- Risk of coronary artery damage, perhaps mediated by host antibody
- 2127 transplants in 2005

**Kidney**
- From live donor or cadaver
- ABO and HLA matching useful
- Immunosuppression usually required
- Risk of GVHD very low
- 16,477 transplants in 2005

**Liver**
- From cadaver
- Surgical implantation complex
- Resistant to hyperacute rejection
- Risk of GVHD
- 6444 transplants in 2005

**Bone marrow**
- Needle aspiration from living donor
- Implanted by IV injection
- ABO and HLA matching required
- Rejection rare but GVHD a risk
Clinical Transplantation - 3

- **Kidney Transplantation**
- Most common (>10⁴/ year in USA).
- Patient survival after one year is expected to be better than 90%.
- The current likelihood of graft function at one year exceeds 85% even when organs from totally unrelated donors are used.
- Subsequent risk of loss to rejection is 3-5% in each subsequent year.
- Choice among multiple donors should be based on MLC [weak (90%) Vs strong (60%) survival in 1 haplotype matched]
## One year kidney graft function*

<table>
<thead>
<tr>
<th>Type of Graft</th>
<th>1976</th>
<th>1986</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-identical grafts (living-related)</td>
<td>90%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>One-haplotype matched grafts (living related)</td>
<td>78%</td>
<td>92%</td>
<td>94%</td>
</tr>
<tr>
<td>Cadaver-donor grafts</td>
<td>58%</td>
<td>83%</td>
<td>86%</td>
</tr>
</tbody>
</table>

* Data from the Transplantation Unit, Massachusetts General Hospital, Boston
• Bone Marrow Transplantation – 1
• The transplantation of pluripotent hemoatopoietic stem cells (Allogeneic Vs autologous).

• It is general practice to transfer stem cells as part of an inoculum of total marrow cells collected by aspiration.

• However, treatment of donor with G-CSF can mobilize stem (CD34+) cells which are then isolated from peripheral blood.

• After transplantation, stem cells repopulate the recipient bone marrow with their differentiating progeny.
Bone Marrow Transplantation - 2

• Recipient must be nearly ablated to permit successful BMT (radiochemotherapy)

• Complications:
  1) venoocclusive disease of the liver
     - 20%, due to high doses of chemoradiotherapy.
     - 8-20 days after transplantation.
     - Fatal in 5-20%, resolves in 60% with no effective treatment.
     - Hepatitis is a risk factor.
Clinical Transplantation - 6

• Bone Marrow Transplantation - 3

• Complications:

2) GVHD: usually against minor antigens
   a) **Acute**: epithelial cell necrosis in skin, liver (biliary not hepatocytes), and GI tract causing skin rash, jaundice, diarrhea and GI hemorrhage.

   b) **Chronic**: characterized by fibrosis and atrophy of one or more of the same organs without evidence of acute cell necrosis.

3) Clinical Immunodeficiency
Acute GVH
• Acute graft-versus-host reaction with vivid palmar erythema
Liver Transplantation - 1

- A major technical challenge (esp. size).

- Successful liver transplantation can now be achieved with survival of about 2/3 of recipients at one year.

- The organ is apparently highly resistant to immediate Ab - mediated rejection (successful at the short term in face of a positive cross match but long-term survival seems to be influenced).
Liver Transplantation - 2

- Long-term survival does not appear to be better when HLA matching is achieved.

- Rejection defined by histologic means is common (75%) but it is easily reversed and does not influence long-term survival.

- Living related liver lobe transplantation is now commonplace.
Problems of Transplantation

• There are not enough organs
  – At least 150,000 patients in industrially developed countries badly need donor organs and tissues
  – Every 14 minutes another name is added to the national transplant waiting list.
  – About 16 people die because of the lack of available organs for transplant each day.

• Rejection:
  – When the immune system of the host detects foreign graft tissue, it launches an attack, resulting in tissue rejection
Gene technology as a solution

- Gene technology offers the possibility to breed the desired organs in animals: **Lack of organs is no longer a problem**

- Gene technology makes it possible to humanize the bred organs; the immune system identifies the organ as its own tissue: **Immune system rejection is prevented**
From which animals are we able to transplant organs?

1. The Chimpanzee: Its DNA sequence differs from ours by only 2%
2. The Baboon: Its organs are too small for a large adult human
3. The Pig: Surprisingly similar to our anatomy and physiology
Organ breeding

• A transgenic animal carries a foreign gene inserted into its genome.
• The transgenic animal shows the specific characteristics which are coded on the inserted gene.
• A gene which is responsible for the construction of a human organ makes the organism produce the organ additionally.
The insertion of a foreign gene into an animal

I. DNA microinjection
The DNA is inserted into the cell with a small syringe

II. Retrovirus gene transfer
The DNA is carried into a cell by a virus.