Transplantation

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Outline

- Scope of Transplantation
  - Allogeneic
  - Autologous
- Allogeneic Solid Organ Transplantation
  - Hyperacute Rejection
  - Acute Rejection
  - Chronic Rejection
- Xeno Transplantation
- Alternative/Stabilizing Devices
- Allogeneic Stem Cell Transplantation (aSCT)
- Future and Needs of aSCT

Self non-Self Boundaries and Transplantation Applications

Allogeneic (Non-self Donor)

- Solid Organ transplants
  - Correct organ failure
    - Kidney
    - Liver
    - Heart
    - Pancreas
    - Lung
    - Small Intestine
- Tissues
  - Restore Function/Costmetic
    - Face
    - Limb
  - Correct endocrine defect
    - Pancreatic Islets
- Stem Cell Transplant
  - Genetic diseases
  - Hematological Malignancy
  - Bone marrow failure

Autologous (Self, Identical Twin)

- Organs
  - Identical twin donor (Joseph Murray, 1990 Nobel Prize)
- Tissues
  - Skin
    - Treatment for burns
  - Hair follicles
    - Male pattern alopecia
- Stem Cell Transplant (misnomer?)
  - Allows high (ablative) doses of chemo to patient
  - Allows ex-vivo purging of cancer cells
  - Ex-vivo gene therapy to correct single gene defects

Clinical Organ Transplantation in U.S.

Organ	Number (2004)	Graft Survival (%) 1 Yr	5 Yr	10 Yr
Kidney (Deceased)	9025	89.0	66.7	40.5
Kidney (Living)	6646	95.1	80.2	56.4
Liver (Deceased)	5457	86.8	66.9	52.5
Heart	1961	85.3	71.8	51.1
Pancreas	1429	85.8	71.0	53.6
Lung	1168	81.4	47.5	22.1

Data from 2005 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients. Cohorts are transplants performed during 2002-2003 for 1 year rates; during 1998-2003 for 5 year rates, and 1993-2003 for 10 year graft survival rates. Graft survival rates shown for pancreas transplants are those for kidney-pancreas transplants.

Allogeneic Transplantation

Solid Organ

- Organs
  - Kidney
  - Liver
  - Heart
  - Pancreas
  - Lung
  - Small Intestine
- Tissues
  - Cornea
  - Tachea
  - Face
  - Limb
  - Pancreatic Islets

Blood Cells (plus…)

- Blood transfusion
- Platelet transfusion
- Stem Cell Transplantation
  - Peripheral Stem Cells
    - T cells
  - Bone Marrow
    - T cells
- Dendritic Cells plus Organs
  - Inadvertent
  - Key to chronic

Donor dendritic and stem cells in solid organ transplants

- Incidental donor dendritic and stem cells are transplanted with grafts
  - Mixed allogeneic chimerism (particularly of donor dendritic cells) observed by Starzl and others post-hoc (XX, XY, etc.)
  - Long term surviving solid organ transplant recipients have donor dendritic cells throughout the body
- Pre-treatment with donor bone marrow cells
  - Future of transplantation: tolerance without drugs
  - Megan Sykes (Columbia) is pioneer
  - Limited to living related donor (kidney

Successful allo-Transplants

- Successful transplantation exploits
  - Plasticity of immune system
  - Regenerative capacity of immune system
- Origins of plasticity and regenerative capacity
  - Ontogeny
    - Immune system develops anew in each individual
    - Shaped by unique complement of paternal and maternal HLA and minor histocompatibility Ags
  - Pregnancy
    - Pregnancy is a tumor of mixed allo-halplotype
    - Parturition (delivery) breaks immune privilege of placenta
- Goal of medical support of transplant
  - Temporize until plasticity and regenerative

Types of Rejection

- Hyperacute
  - Cause: Pre-formed antibodies against ABO or HLA antigens (from pregnancy, blood transfusion or previous transplants)
  - Solution: Matching donor/recipient and screening for pre-formed antibodies
- Acute
  - Cause: Effector T cells responding to HLA differences between donor and recipient
  - Solution: Matching donor/recipient for HLA and treating recipient with immunosuppressive agents
- Chronic
  - Cause: Multi-factorial
  - Solution: unsolved

Hyperacute rejection

Why

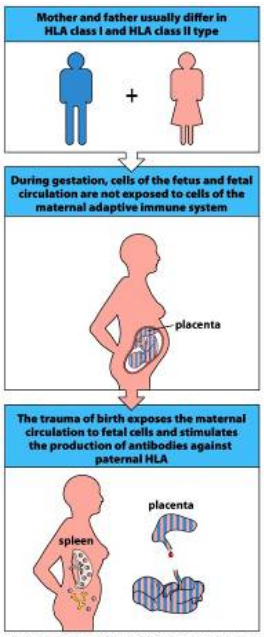


Figure 15.4 The Immune System, 3ed. (© Garland Science 2009)

How

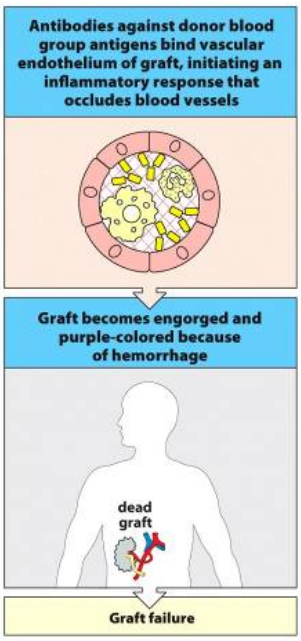


Figure 15.3 part 2 of 2 The Immune System, 3ed. (© Garland Science 2009)

Mechanism of Acute Rejection

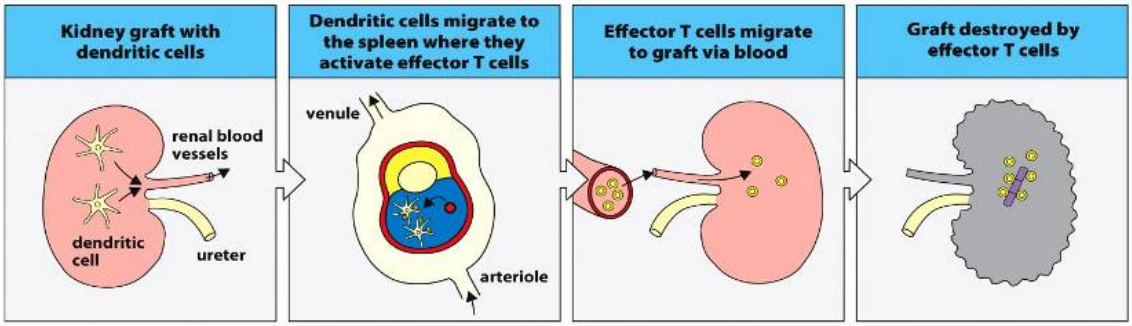


Figure 15.7 The Immune System, 3ed. (© Garland Science 2009)

- Donor dendritic cells stimulate T effector cells to reject the graft
- However, donor dendritic cells ultimately MAY help permit "détente" if not tolerance
  - Delete or anergize donor's graft specific T cells?
  - Stimulate T_{reg}?

Screening to prevent Acute Rejection

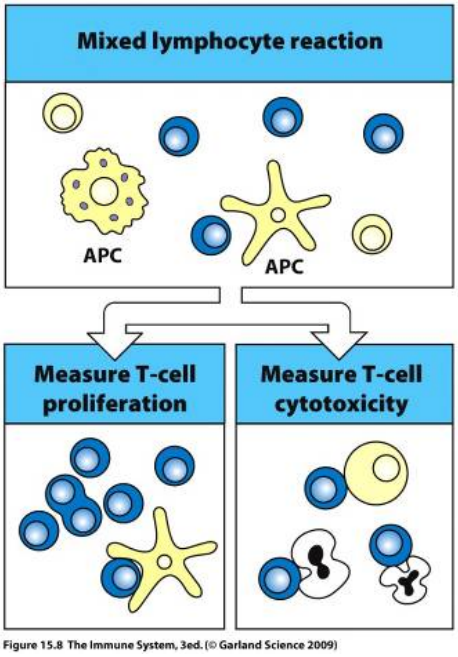


Figure 15.8 The Immune System, 3ed. (© Garland Science 2009)

Treatment Philosophy

- Immunosuppressive drugs predispose to infection (and neoplasia)
  - At the time of transplant, acute rejection is prevented by high doses of immunosuppressive drugs
  - High doses of Cyclosporin result in polyclonal B cell neoplasia
- As doses are decreased, episodes of acute rejection (varying degrees of severity) occur periodically in transplant recipients
  - Some find comparisons between episodes of rejection and flares (exacerbations) of autoimmune diseases
  - To extend that metaphor, transplantation

Treatments for Acute Rejection

- Corticosteroids
  - a.k.a glucocorticoids
  - Modulate gene expression
- Cytostatic Drugs
  - Mycophenolate mofetil (MMF)
  - Azathioprine
- Cytotoxic Drugs
  - Cyclophosphamide
- Anti-T cell antibodies
  - Polyclonal anti-thymocyte globulin
  - mAb anti-CD3 (TCR complex)
  - mAb anti-IL2R (CD25)
  - mAb anti-CD52 (pan-lymph)
- Calcineurin Inhibitors
  - Cyclosporin A
  - FK506/tacrolimas
- mTor inhibitor

Corticosteroids

Corticosteroid therapy	
Activity	**Effect**
↓ IL-1, TNF-α, GM-CSF ↓ IL-3, IL-4, IL-5, CXCL8	↓ Inflammation ↓ caused by cytokines
↓ NOS	↓ NO
↓ Phospholipase A_2 ↓ Cyclo-oxygenase type 2 ↑ Lipocortin-1	↓ Prostaglandins ↓ Leukotrienes
↓ Adhesion molecules	Reduced emigration of leukocytes from vessels
Induction of endonucleases	Induction of apoptosis in lymphocytes and eosinophils

Figure 15.16 The Immune System, 3ed. (© Garland Science 2009)

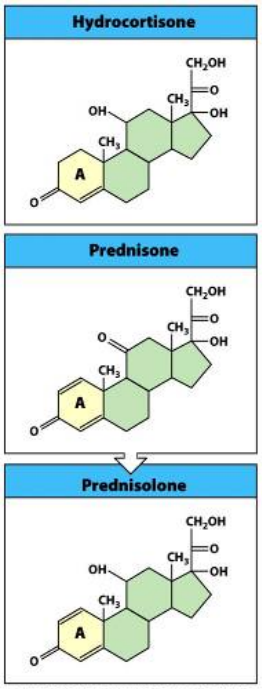


Figure 15.14 The Immune System, 3ed. (© Garland Science 2009)

Cytostatic and Cytotoxic Drugs

Targeting cell division (proliferating cells) targets lymphocytes, because proliferation (clonal expansion) is a specialized function of lymphocytes

- Purine analogues
  - Azathioprine and 6-mercaptopurine
  - Not FDA approved for transplant
- Purine synthesis inhibitor
  - Mycophenolate mofetil (MMF,Cellcept®)
  - Mycophenolate sodium (Myfortic®)
- Alkylating agents
  - Cyclophosphamide
  - Not FDA approved for transplant
- Folic Acid analogue

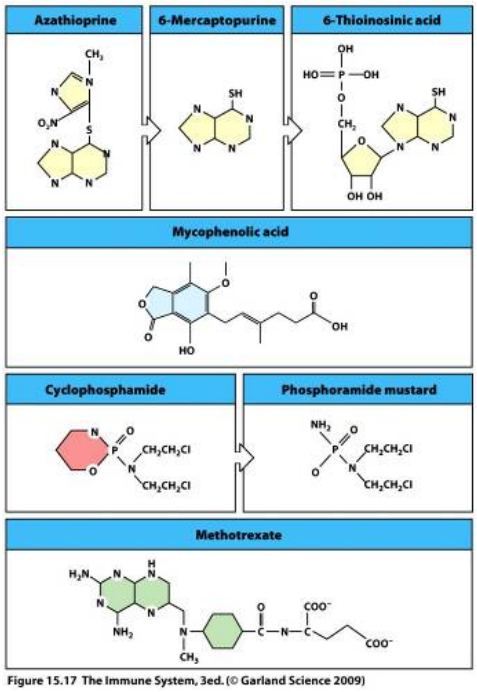


Figure 15.17 The Immune System, 3ed. (© Garland Science 2009)

anti-T cell antibodies (I)

- Poly-clonal anti-thymocyte globulin
  - Used to Tx acute rejection
  - rabbit (Thymoglobulin®)
  - Horse (Atgam®); (Lymphoglobulin) – off market
- mAb anti-CD3 (TCR complex)
  - Expressed on resting and activated T cells
  - Murine mAb (muromonab; Orthoclone®)(Paul Russell, P&S)
  - First mAb approved by FDA
  - Used for acute rejection

anti-T cell antibodies (II)

- mAb anti-IL2R (anti-tac, anti-CD25)
  - Expressed on activated T cells; block IL2 signaling
  - Chimeric mu/hu mAbs against the alpha chain of IL2R
    - Basiliximab (simulect®)
    - Daclizumab (Zenapax®)
- Anti-CD52
  - Expressed on most lymphocytes
  - alemtuzumab (Campath®) is a depleting murine mAb
  - not FDA approved for transplant

Cyclosporin and Tacrolimas have similar downstream effects

- Cyclosporin and Tacrolimas (FK506) bind to different targets but have similar downstream effects
  - Calcineurin Inhibitors (CNIs)
  - Inhibit T cell activation
- Targets
  - Cyclosporin: cyclophilin (CyP)
  - Tacrolimas: FK binding protein (FKBP)
- Cyclosporin-CyP and Tacrolimas-FKBP both bind to and inhibit Calcineurin
  - Neither CyP nor FKBP normally bind Calcineurin, but are believed to be peptide isomerases
  - Cyclosporin is a cyclic peptide; Tacrolimas is a macrolide

CNI mechanism I

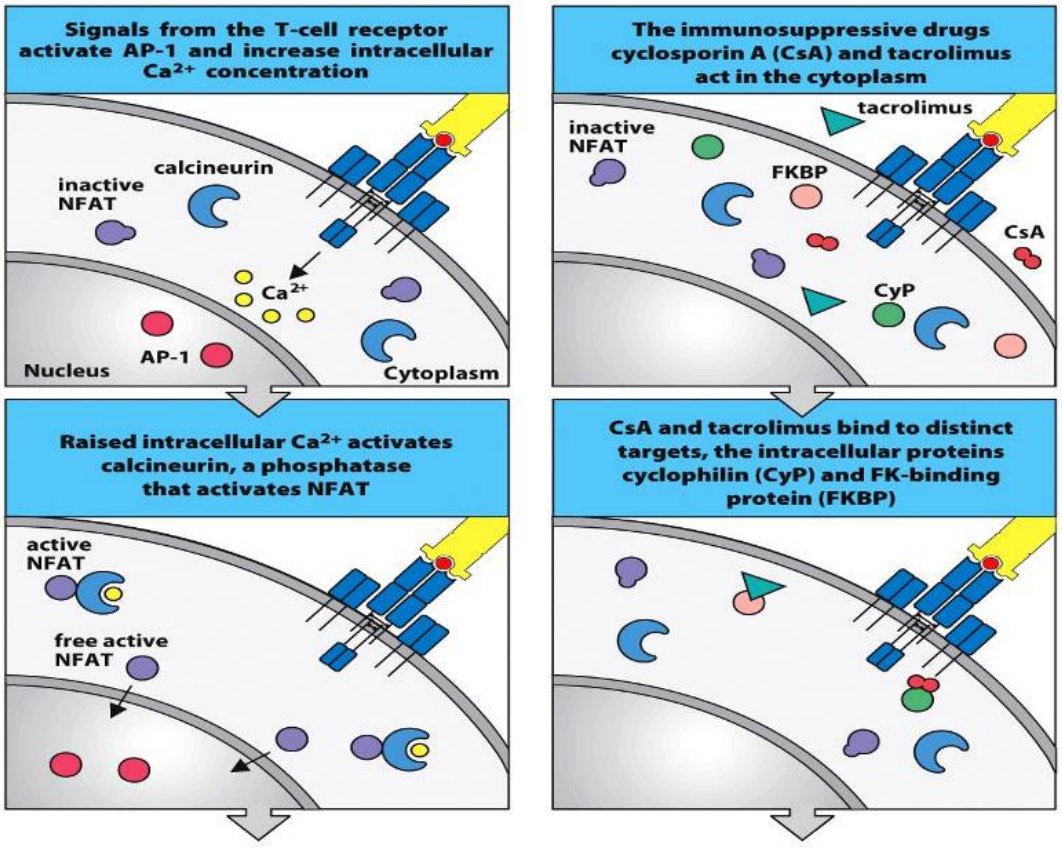
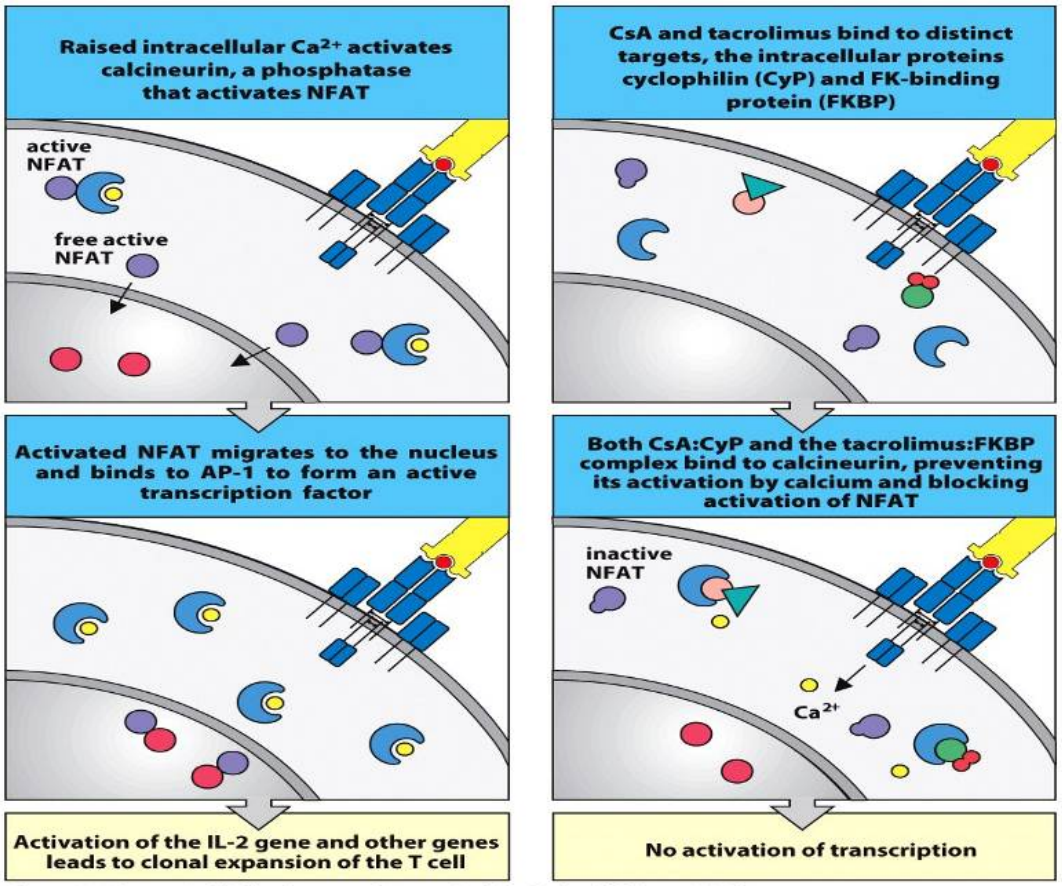


Figure 15.18 part 1 of 2 The Immune System, 3ed. (© Garland Science 2009)

CNI mechanism II



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CNIs have similar treatment effects

Cell type	Effects of cyclosporin A and tacrolimus
T lymphocyte	Reduced expression of IL-2, IL-3, IL-4, GM-CSF, TNF-α Reduced cell division because of decreased IL-2 Reduced Ca^{2+}-dependent exocytosis of cytotoxic granules Inhibition of antigen-driven apoptosis
B lymphocyte	Inhibition of cell division because T-cell cytokines are absent Inhibition of antigen-driven cell division Induction of apoptosis after B-cell activation
Granulocyte	Reduced Ca^{2+}-dependent exocytosis of granules

Figure 15.19 The Immune System, 3ed. (© Garland Science 2009)

Rapamycin binds FKBP but interacts with mTOR (not Calcineurin)

- Rapamycin (aka sirolimas) binds FKBP
  - Rapamycin:FKBP complex binds “mammalian target of Rapamycin” (mTOR)
  - Rapa binds the same FKBP as Tacrolimas, but induces interaction with a different molecular target (mTOR instead of calcineurin)
  - Rapa:FKBP inhibits mTor complex and pathway
  - Rapa:FKBP:mTOR inhibits the production of IL2 (whereas CNI’s inhibit IL2 production)
- Used in kidney transplant and has safety advantage in patients with uremia

Co-evolution of Transplantation Clinical and Basic Science

- Effective drugs elucidate complex biology
  - Cyclosporin, Tacrolimas (FK506) and Rapamycin were used to identify and characterize their respective molecular targets (Cyclophilin, FKBP and mTOR) AFTER they were either approved for use or well advanced in clinical testing
- Kidney Transplantation was already established with Prednisone/Azathioprine before the era of CNIs

Chronic Rejection (CR)

- Primary lesions
  - Vascular endothelium (intimal proliferation)
  - Graft parenchyma (fibrosis) with loss of normal structure
- Treatment Phenomena
  - More likely following acute rejection
  - More intensive T-cell depletion doesn’t prevent CR
  - MMF, Rapa delay but don’t prevent CR

Chronic Rejection: Indirect Recognition

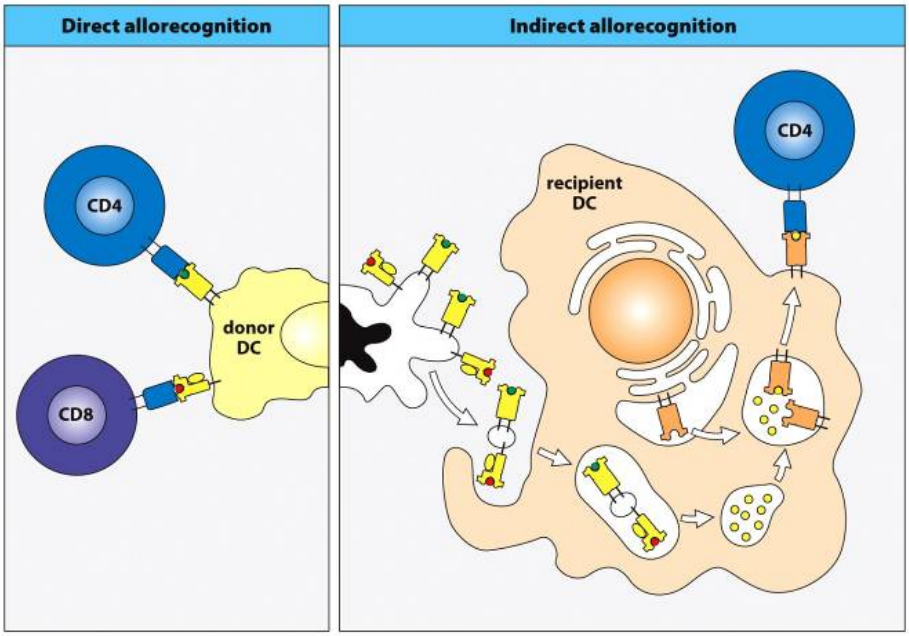

Figure 15.11 The Immune System, 3ed. (© Garland Science 2009)

Chronic Rejection: Alloantibodies

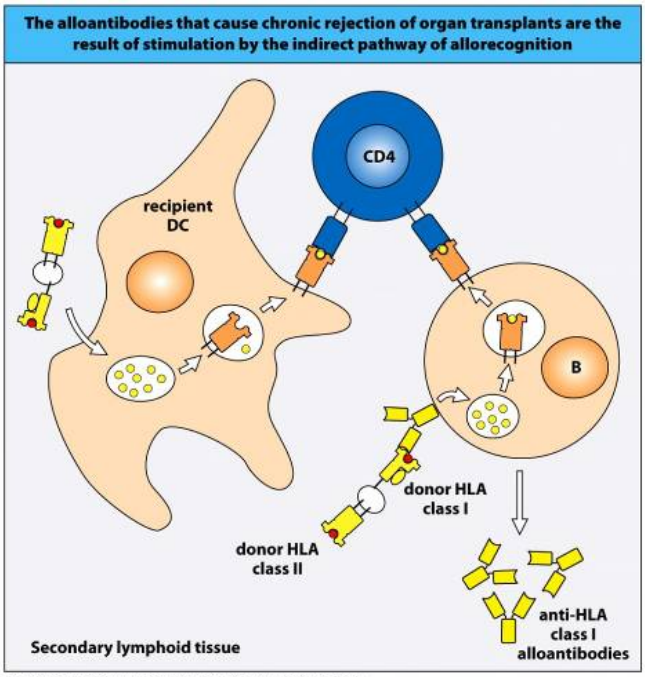

Figure 15.12 The Immune System, 3ed. (© Garland Science 2009)

Chronic Rejection: New Targents/New Approaches

- CD40-L Helper Function
  - mAb anti-CD40L has unique activity (heart, islets) but may have been associated with thrombosis in human SLE trial (Lederman)
  - Concept is to targeti activated T helper cells “in flagrante delicto” to spare resting cells
  - Second generation, single chain anti-CD40L designed to reduce toxicity
- Promote T-regulatory cells to protect the graft (peripheral tolerance)
  - $CD8^{+}CD28^{-}$ T suppressor (Ts) cells: ILT3 and ILT4 on dendritic cells and other APC may stimulate $CD8^{+}$ Ts cells (Nicole Suciu-Foca)
  - $CD4^{+}CD25^{+}FoxP3^{+}$
- B-cells (source of allo-antibodies)?
  - Deplete B cells (with mAbs) for induction therapy
- Chemokine receptor inhibition?
  - Block chemokines (CCR5) associated with macrophages
- Fibroblasts?
  - Block proliferation of fibroblasts

Graft Chimerism: Good or Bad?

- Grafts are repopulated to some extent by host bone-marrow derived stem cells
  - Normal process by which organs are replenished
  - Normally organs are also replenished by tissue-derived stem cells (which may operate in grafts)
- Inflammation is a trigger for bone-marrow derived stem cells to home and differentiate
  - Tim Wang (Columbia) has shown that inflammation recruits stem cells to stomach that have the potential to become epithelial (adenomatous) cancers
  - Some evidence suggests graft chimerism (with host cells) is associated with

Ethical Issues

- Should donors (or their survivors) be paid?
- What is acceptable “pressure” on healthy potential donors?
  - Donation of partial or whole organs carries risks
- Should alcoholics be allowed to die of treatable diseases?
  - When is an alcoholic not an alcoholic?
- Cost – expensive procedures
- Organ supply requires triage
  - Decisions of relative merit between “worthy” patients
- Preference to “right” patient

Xenotransplantation?

Xenograft: Transplant between species

- Concordant: No hyperacute rejectio
  - Primate-to-Man
  - Problems: Ethics, supply, infection (SIV)
- Discordant: Hyperacute rejection
  - Pig-to-Man
  - Problems: Scientific (infection? PERV)

Problems and Potential Solutions to Pig Xenografts

- Pig cells express Gal 1,3 αGal sugars to which humans have pre-formed antibodies
  - Solution: Generate Gal-transferase knock-out [KO]Pigs
- Human complement is activated by other pig proteins
  - Solution: Generate Human CRP (human complement regulatory protein) – transgenic [tg] pigs
- Work on Gal-transferase[KO]; CRP[tg] pigs is ongoing

Devices: Temporizing Measures or potential Solutions

- Kidney Dialysis
  - Can be long term solution
    - Hemodialysis
    - Peritoneal dialysis
- Left Ventricular Assist Device (LVAD)
  - Supports heart failure patients waiting transplant
- Ex-vivo Liver Assist Devices (Not FDA Approved)
  - Temporizes patients waiting transplant; allows some fulminant hepatic necrosis patients to recover
    - Bio-artificial liver

Tissue Engineering

- Autologous tissues grown ex-vivo could be substitutes for transplants
  - Nose
  - Ear
  - Liver?

Allogeneic Stem Cell Transplantation (aSCT)

- High level of specialized skills and facilities required
  - Not commonly performed
  - Donnall Thomas, 1990 Nobel Prize (P&S Resident)
- Goal
  - to transplant hematopoietic stem cells and achieve engraftment and chimerism
- Peripheral Blood Stem Cell Transplant (aPBSCT) is replacing Bone Marrow Transplant (aBMT)
  - Faster engraftment (reduces fungal infections)
    - aPBSCT engraft 14-17 d
    - aBMT engraft 21-24 d
    - Risk of fungal infections begins week 2 of neutropenia

Obtaining Allogeneic Stem Cells

- Donors
  - Identified by screening
    - Relatives
    - Matched Unrelated Donor (MUD) pool
    - Umbilical cords
  - Mobilization of stem cells by GCSF or GMCSF
  - GCSF/Plerixafor (Mozobil®/CXCR4 antagonist) FDA approved for autologous SC mobilization (NOT FDA approved for ALLO)
- Peripheral stem cells
  - Apheresis/Leukophoresis for 4 hours/day (1-4 days) during which CD34+ cells are collected (Minimum 2 x 10^6/kg)
- Bone marrow
  - Painful harvesting and several days recovery
  - Fall-back if donor is a "poor-mobilizer"

Achieving engraftment and survival in aSCT: Challenges/approaches

- Engraftment
  - Preparative Chemo/Radiation
- Rejection (allo-reactivity)
  - Immunosuppression
- GvH (Graft versus Host) allo-reactivity
  - HLA Class I and II matching
  - Deplete mature T cells from graft
  - Immunosuppression (e.g. corticosteroids, CNIs, IVIG, MMF)
- Infection
  - Antibiotics and anti-fungals
- Malignant clones (in recipient's

Uses of aSCT

Replace dysfunctional BM

- Correct genetic defect
  - Hemaglobinopathies
- Correct bone marrow failure
  - Aplastic anemia

Treat malignant disease

- Replace bone marrow
- Eradicate malignant clone
  - Malignancies/Pre-Malig.
    - Hodgkin’s Lymphoma
    - Leukemia
      - AML
      - ALL
      - CML
    - Multiple Myeloma
    - Myelodysplasia
    - Non-Hodgkin’s Lymphoma (NHL)

Preparation and Treatment of PSC grafts and aPSCT Patients

Replace dysfunctional BM

- Preparative chemotherapy may not need to be myelo-ablative
- Mature T cells depleted from graft (to limit GvH)
- Immuno-suppression needs to be potent so that graft isn't rejected

Treat malignant disease

- To eradicate malignant clone, preparative chemo is typically ablative (w/ cytoreduction)
- Mature T cells may not be completely depleted from graft (GvH is tolerated to preserve graft v. tumor effect)
- Immuno-suppression may be moderated because rejection risk is lower (host immune system is ablated) and graft v.

Clinical Graft v. Host Disease (GvH)

Tissue reactions in the four grades of graft-versus-host disease

Grade	Skin	Liver	Gastrointestinal tract
I	Maculopapular rash on <25% of body surface	Serum bilirubin 2–3 mg/dl	>500 ml diarrhea/day
II	Maculopapular rash on <25–50% of body surface	Serum bilirubin 3–6 mg/dl	>1000 ml diarrhea/day
III	Generalized erythroderma	Serum bilirubin 6–15 mg/dl	>1500 ml diarrhea/day
IV	Generalized erythroderma with blistering and desquamation	Serum bilirubin 15 mg/dl	Severe abdominal pain with or without intestinal obstruction

Figure 15.27 The Immune System, 3ed. (© Garland Science 2009)

- GvH is caused by mature T cells in graft
  - Better matching means less need to deplete T cells
  - Some GvH is desirable in eradicating malignant clones
    - Currently GvH activity cannot be separated from Graft v. tumor activity

Genetic diseases treatable by bone marrow transplatation	
Disease	**Deficiency**
Wiskott–Aldrich syndrome	Defective leukocytes and platelets
Fanconi anemia	Failure of bone marrow to make blood cells
Kostmann syndrome	Low neutrophil count (neutropenia)
Osteopetrosis	Defective bone modeling and remodeling by osteoclasts
Ataxia telangiectasia	Neurological impairment and immunodeficiency
Diamond–Blackfan syndrome	Low erythrocyte count (anemia)
Mucocutaneous candidiasis	Ineffective T-cell response to fungal infections
Cartilage–hair hypoplasia	Short limbs, fine sparse hair and immunodeficiency
Mucopolysaccharidosis	Various deficiencies of lysosomal enzymes
Gaucher's syndrome	Deficiency of the lysosomal enzyme glucocerebrosidase
Thalassemia major	Defective hemoglobin, impared erythrocyte function
Sickle-cell anemia	Defective hemoglobin, impaired erythrocyte function

Figure 15.24 The Immune System, 3ed. (© Garland Science 2009)

HLA Matching Influences Survival

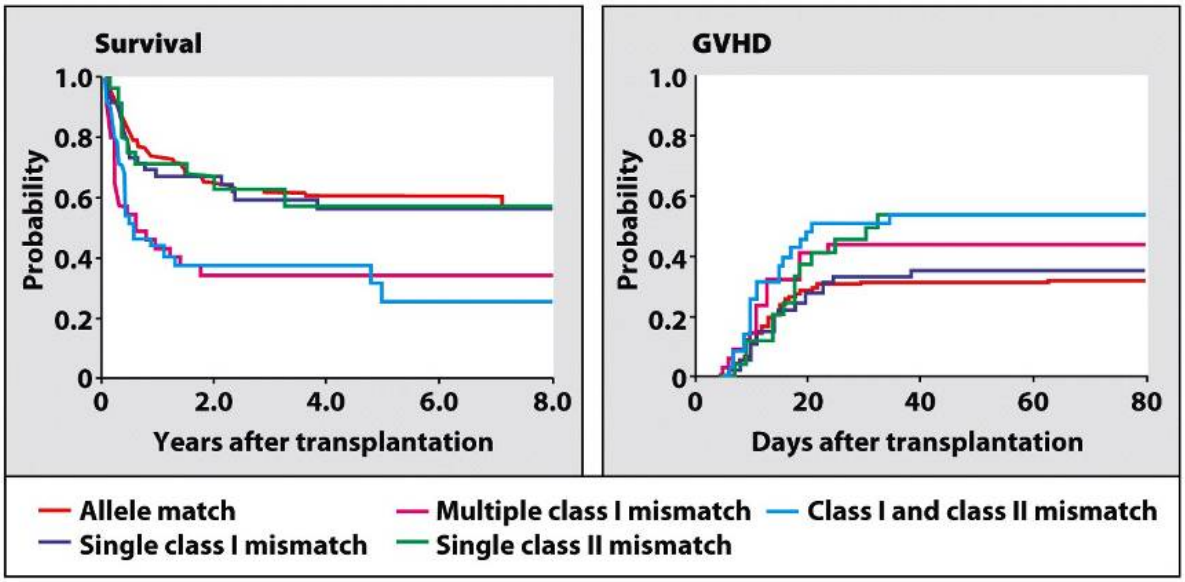

Figure 15.28 The Immune System, 3ed. (© Garland Science 2009)

Tradeoffs:

Survival (disease-free, 5 years) in Intermediate prognosis AML – First Remission; <60 yo's is approximately 50%

Autologous Transplant

- ~40% Relapse
- ~10% Die of transplant related complications

Err on side of relapse?

- Donor disparity
- Co-morbidity

Allogeneic Transplant

- ~10% Relapse
- ~40% Die of transplant related complications

Err on side of allo-transplant?

- All matched
- Young & healthy

Need better markers to provide data to decide on risk adjusted strategies

These may come from molecular profiling of tumors

Future of aSCT

- Use of aSCT is decreasing
  - Imatinib (Gleevec®) and second generation BCR-Abl kinase inhibitors (nilotinib [Tasigna®], and dasatinib [Sprycel®]) are treating many CML patients who used to be transplanted
  - Gene therapy (and autologous transplant of transduced SC) for certain diseases will replace aSCT
    - But risks associated with transgene, promoter and other factors need to outweigh risks of aSCT for adoption
- Use of aSCT may increase
  - Preparation for solid organ transplant
    - Elegant immunological solution, so far limited to elective/non-emergency situations and with living related donors (e.g., kidney, partial liver)
  - Increase size and diversity of donor network

Needs for research in aSCT

- Separate graft v. tumor activity from GvH activity
  - Specific T cell clones?
- Molecular profiling of tumors to provide risk adapted strategies for each patient
- Purification of stem cells within CD34+ population
- Drugs to increase efficiency of tri-lineage engraftment
  - Valproate?

Feedback & Acknowledgements

- Questions/comments:

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  - Michael Bar, MD – Asst. Clin. Prof. of Medicine, Bennett Cancer Center at Stamford Hospital (Heme/Onc)
- Citations
  - Suciu-Foca: J Immunol. 2006 Mar 1;176(5):2790-8.