Basic Transplant Immunology & Immunosuppressive Drugs

Luis S. Marsano, MD
Professor of Medicine
Division of Gastroenterology, Hepatology & Nutrition
University of Louisville &
Louisville VAMC

Immune System

- Immune system:
  - protects against infectious invasion, and
  - provides self-nonself discrimination.
- It has two sub-systems:
  - Rapid response: done by innate immunity system.
  - Specific response: classic T-cell response requiring days to weeks; this T-cells mediate graft rejection but also tolerance.
- Both sub-systems are coordinated and provide immunological memory.

Hyperacute Rejection

- Extremely rare.
- Occurs hours to days after transplantation,
- Target is vascular endothelium.
- Antibody-mediated, & complement dependent graft destruction by ***coagulative necrosis***.
- Preformed antibodies specific to MHC.
- Lack of lymphocytic infiltration.

Acute Rejection

- Occurs in 45-70% of patients.
- Days to months after transplant (usually initial 3 months).
- Classical, cell-mediated rejection:
  - Predominantly CD4 & CD8 T-cells.
  - Directed against donor MHC antigens (cholangiocytes & vascular endothelium)
- Target of current immunosuppression.
- Diagnostic Triad:
  - Portal inflammation
  - Bile-duct damage
  - Venular endothelium inflammation.

Risk of Rejection in OLTx with Different Regimens

Regimen	Acute (ACR)	Chronic (Ductopenic)
Pred + Aza	85 %	25 %
Pred + CyA	70 %	15 %
Pred + Tacr	55 %	6 %
Pred + CNI + MMF	45 %	1 %
Tacr + Rapa	18 %	1 %

Chronic Rejection

- Occurs to 2-5% of patients.
- Slow, indolent process months to years after transplantation.
- Has immune & non-immune components; poorly defined.
- Causes ischemic injury and paucity of bile ducts.
- Characterized by arteriole thickening & interstitial fibrosis.
- ***Loss of small bile ducts*** +/- neo-intimal proliferation with ***obliterative vasculopathy***.

T-cell Recognition of Alloantigen & T-cell Activation: Rejection

- Recipient T-lymphocytes recognize a donor alloantigen by:
  - a) ***Direct Path*** : native donor ***MHC*** molecule expressed ***in donor APCs*** **,**
  - b) ***Indirect Path*** : ***donor alloantigen peptides*** (from damaged cells or soluble MHC class I) ***presented by recipient APCs***.
- "Direct path" dominates in "acute" rejection, and
- "Indirect path" in chronic rejection and tolerance.

Costimulatory Pathways & Transplantation: Rejection

- Optimal T-lymphocyte activation need TWO coordinated signals:
  - **Signal 1**: T-cell Receptor (TCR) signal, which occurs after recognition of peptide/MHC on APC, and
  - **Signal 2**: occurs from interaction of "costimulatory T-cell molecule" with its "ligand" on the APC (eg: CD28/B7, CD40/CD154)
- If signal 1 & 2 occur, rejection develops.

Effector Pathways of Graft Injury
Rejection

- There is not a single mediator or cell type that is absolutely required for allograft rejection; there are several redundant and compensatory mechanisms contributing to rejection.
- After [T-cell Receptor signal + costimulatory signal, + cytokines], there is proliferation and maturation of $CD4^+$ or $CD8^+$ T-cells capable of graft injury; this will lead to:
  - T-cell mediated cytotoxicity
  - Delayed hypersensitivity
  - Antibody-mediated damage

Effector Pathways of Graft Injury
Rejection

- **<u>T-cell mediated cytotoxicity</u>**:
  - ***A) CD8+*** cytotoxic T-lymphocytes (CTLs) specific for donor ***class I***, cause apoptosis through biochemical mechanisms (perforin/granzyme B in a Ca^{++} dependent process, and Fas/FasL through caspase 8);
  - ***B) NK cells***, ***without*** need for ***activation*** or sensitization, which can cause apoptosis through FasL & granzyme B.

Effector Pathways of Graft Injury
Rejection

- **Delayed hypersensitivity**:
  - ***CD4*⁺** T-lymphocytes specific for donor ***class II***, release IFN gamma activating macrophages and cellular mediators.
- **Antibody-mediated damage**:
  - Antibodies against liver sinusoidal endothelial cells (LSECs) indirectly promote acute rejection.

Immune System & Tolerance

- **Tolerance**: Absence of destructive response to an allograft in immunocompetent host.
- Tolerance is accomplished by T-cell suppression mediated by :
  - a) **cell-contact dependent mechanism**: $CD4^+CD25^+$ cells,
  - b) **cytokine mediated T-cell mechanism**: T regulatory-1 & T helper-3 (Th3),
  - c) **antigen presentation dependent mechanism**: by liver-derived Dendritic Cells (DCs) and by Liver Sinusoidal Endothelial Cells (LSECs) which behave as immature DCs causing incomplete activation, inhibiting T-cell response.
  - d) **NK cells** which give a “death signal” to recipient derived T-cell passing through the graft.

Costimulatory Pathways & Transplantation: Tolerance

- Optimal T-lymphocyte activation needs coordinated “signal-1” and “signal-2” stimuli.
- If only “signal 1” occurs, tolerance develops;
- To prevent rejection and induce tolerance, you can disrupt “signal 2”:
  - a) CTLA4 (cytotoxic T lymphocyte antigen 4) can compete with CD28 for B7, and gives “negative costimulation”; CTLA4-Ig fusion protein has been used for this goal,
  - b) anti-CD154 disrupts CD40/CD154 pathway.
- Programmed death-1 (PD-1) is a molecule induced upon T-cell activation and causes a “negative signal” similar to CTLA4, causing spontaneous tolerance. PD-1 binds to ligands PDL-1 & PDL-2.

Possible Mechanisms for Liver Tolerance

- 1) The liver produces large amounts of soluble MHC class I antigen, causing:
  - a) Passive blockade of alloantibodies & donor-specific effectors, or
  - b) Activation-induced apoptosis of allospecific CTLs.
- 2) Liver suppressor factor-1: is produced by spontaneously tolerant recipients and prolongs rat cardiac allograft survival.
- 3) Liver produces a soluble Fas “incomplete variant”, which inhibits anti-Fas induced apoptosis and inhibits CTL function in vitro.

Possible Mechanisms for Liver Tolerance

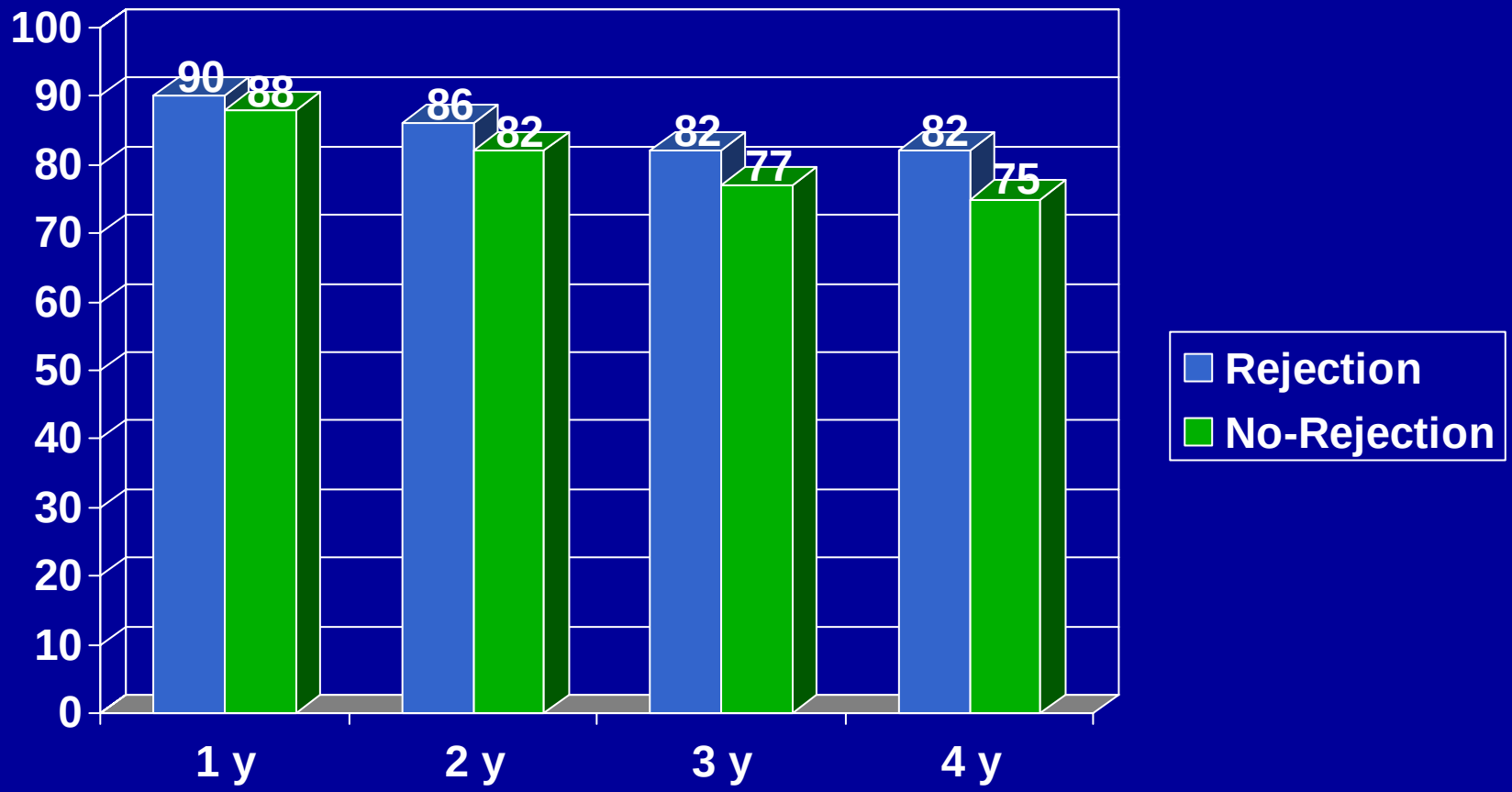
- 4) Graft-derived Stem-cells migrate out of the liver and establish “microchimerism” with clonal exhaustion/deletion of host alloreactive T-cells.
- 5) Inmature “Dendritic Cells” (DCs) and “Liver Sinusoidal Endothelial Cells” (LSECs) do not express enough costimulatory molecules, hence facilitate tolerance.
- 6) Kupffer cells (APCs) express FasL which can induce apoptosis of host T-cells.

Costimulatory Pathways & Transplantation: Autoimmunity

- Deficiency in “Programmed death-1” (PD-1) molecule and/or PDL-1 causes autoimmune disorders and autoimmune hepatitis with large amounts of CD8 T-cells in the liver.

Immunosuppression in Liver Transplantation

Survival vs Rejection in OLTx



Rejection/No-Rejection RR = 0.7

Causes of Late Liver-Graft-Loss

Rejection	**< 5 %**
De-novo Malignancy	**15 %**
Infections	**16 %**
Cardiovascular Disease	**20 %**
Recurrent Disease	**35 %**

Excessive Immunosuppression causes more problems than rejection

Impact of ACR Therapy on Survival

Patient	Therapy	RR Mortality
Non-HCV	Steroids	0.5
HCV	Steroids	2.9
HCV	OKT3	5.4

DO NOT TREAT MILD REJECTION IN HCV

Long-Term Complications of Immunosuppression

Renal Dysfunction	**80 %**
Hypertension*	**70 %**
Hyperlipidemia*	**50 %**
Diabetes Mellitus*	**20 %**
Bone Disease*	**20 %**
Skin Cancer	**40 %**
Lymphoma	**4 %**

* Less if Steroids are withdrawn shortly after 3 months

Cyclosporin A

- From *Tolypocladium inflatum*; approved in 1983. Is calcineurin inhibitor.
- Selective immunosuppression by inhibition of T-cell activation.
- CyA forms complex with cytoplasmic receptor “cyclophilin” and inhibits calcium- & calmodulin-dependent phosphatase calcineurin.
  - Calcineurin is vital for the transcriptional process by which IL-2 and other cytokines are activated, which is needed for T-helper cell mediated graft rejection.

Cyclosporin A

- Currently CyA comes as a microemulsion in lipophilic solvent which is less dependent in bile flow (Neoral, Gengraf).
- CyA is metabolized in the liver by P450-3A pathway.

Cyclosporin A Toxicity

- Nephrotoxicity: can be acute or long term; renal failure in up to 20%; can cause hyperkalemia and hypomagnesemia.
- Hyperlipidemia, hyperglycemia, hypertension, gingival hyperplasia, hirsutism.
- 10-28% may have tremor, peripheral neuropathy, psychoses, hallucinations, motor weakness, or seizures.
- May cause Hemolytic Uremic Syndrome.

Cyclosporin A Dose & Target Levels

- Initial 10-15 mg/kg/d divided q 12h; check trough level after 24 h.
- New data indicates that level 2-h post dose represents better “total exposure”.
- Week 0-2: trough 250-350 ng/mL
- Week 3-4: 200-300
- Week 5-24: 150-250 (850-1400 2h post)
- Week 25-52: 100-200

Drugs that Increase Cyclosporin & Tacrolimus Levels

Calcium Channel Blockers	**Antifungals**	**Macrolide antibiotics**	**Pro-kinetics**	**Miscellaneous**
Diltiazem Nicardipine Nifedipine Verapamil	Fluconazole Itraconazole Ketoconazole Voriconazole Clotrimazole	Clarithromycin Erythromycin Troleandomycin Azithromycin Telithromycin	Cisapride Metoclo_ pramide	Amiodarone Cimetidine Methyl-prednisolone Omeprazole Protease inhibitors **Nefazodone** **Ethinyl estradiol**

Drugs that Decrease Cyclosporin & Tacrolimus Levels

Anticonvulsants	**Antibiotics**	**Herbal Preparations**	**Miscellaneous**
Carbamazepine Phenobarbital Phenytoin Fosphenytoin	Rifabutin Rifampin Rifapentin	St. John's Wort	**Probucol** **Terbinafine**

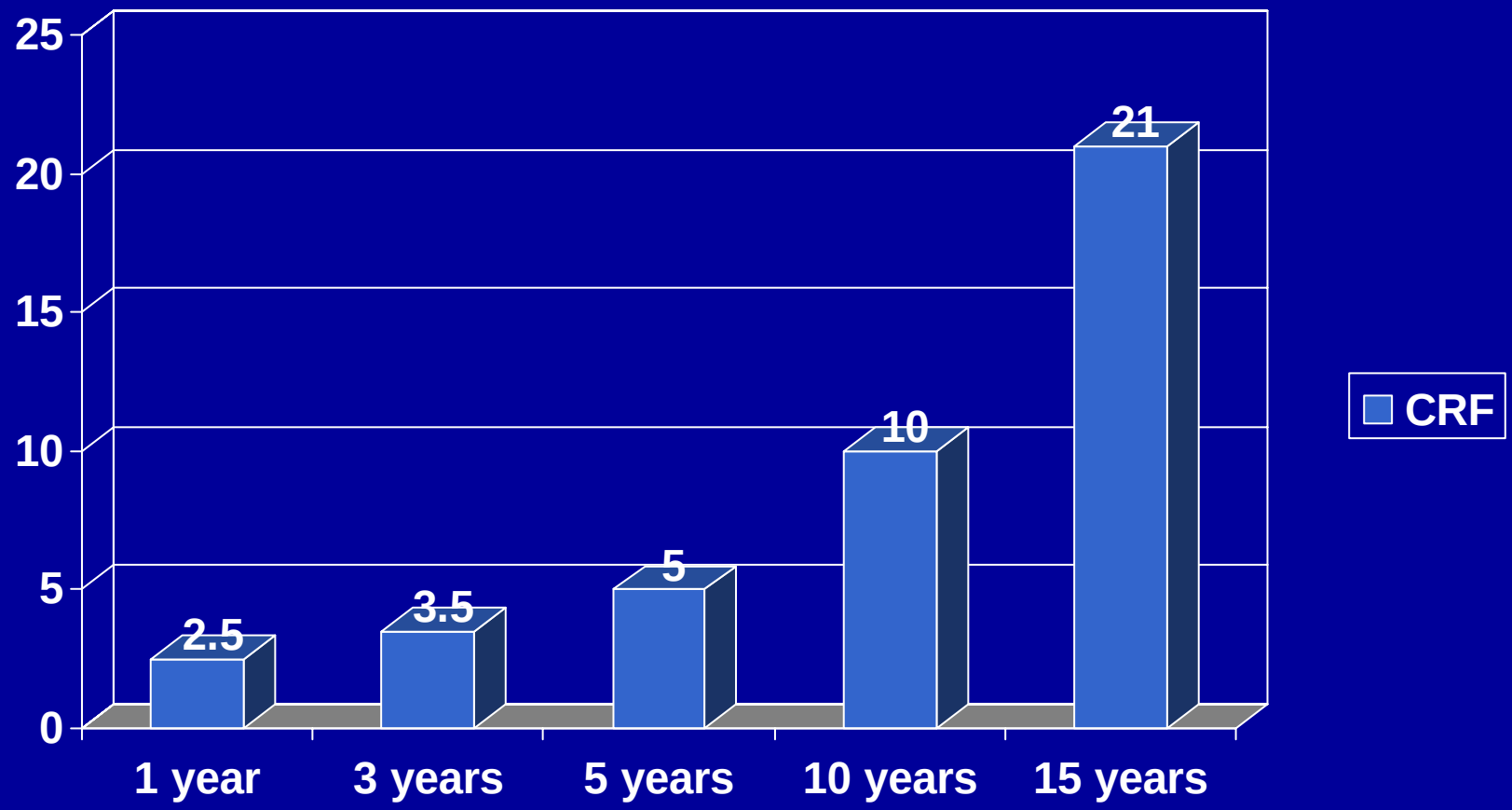
Tacrolimus

- From *Streptomyces tsukubaensis*.
- It is 100-times stronger than CyA.
- Binds to FKBP12 and the complex inhibits calcineurin; this prevents transcription of IL-2, IL-3, IL-4, IL-8, and various chemotactic factors.
- It is absorbed in duodenum & jejunum without need for bile.
- Food decrease bioavailability.
- Metabolized via P450-3A pathway.

Tacrolimus Toxicity & Dose

- More DM than CyA.
- Less HTN, dyslipidemia, hirsutism, gum hyperplasia than CyA.
- Similar hyperkalemia, tremor, hypomagnesemia, infection, malignancies, & renal dysfunction than CyA.
- Nausea, vomiting, diarrhea, headache.
- Less rejection in 1st year in all, less steroid-resistant rejection, and longer graft survival in Hepatitis C than CyA.
- Dose: 0.1-0.15 mg/kg/d divided q 12h p.o.; trough levels 10-15 ng/mL early; 8-10 later.

Calcineurin Inhibitors in OLTx
Risk of Chronic Renal Failure



Risk Factors for CRF in Non-Renal Tx

	Relative Risk
Post-Op ARF	2.13
Diabetes Mellitus	1.42
Age (per each 10 years)	1.36
Hypertension	1.18
Hepatitis C	1.15

Corticosteroids

- Block T-cell-derived and antigen-presenting cell-derived cytokine expression, decreasing IL-1, IL-2, IL-3, and IL-6
- Are used in reversing acute rejection and in maintenance.
- **Side effects**: hypertension, mental status changes, dyslipidemia, poor wound healing, hyperglycemia, gastric ulcers, myopathy, osteoporosis, Cushing S., fungal/bacterial infections, pituitary axis suppression, fluid retention, cataracts.
- Dose: 500-1000 mg pre-op; then taper from 50 to minimal dose over a few months.

Beneficial Effect of Steroid-Withdrawal after 3 months post OLTx

	Steroids	No-Steroids	P-value
Hypertension	58 %	15 %	0.0002
Diabetes	25 %	6 %	0.007
Infection	17 %	2 %	0.05
Bone Disease	9 %	0 %	0.05
Mean Cholesterol	253 mg/dL	183 mg/dL	0.001

Adverse Effects of Steroid-Withdrawal

- Recurrent AIH & PBC
- Worsens HCV if done before 3rd month.
- Flare up of Ulcerative Colitis
- Arthralgias
- Depression

Azathioprine (AZA)

- Antimetabolite; antagonises purine metabolism. Inhibits synthesis of DNA, RNA, and proteins.
- Used in < 5% US transplant centers.
- Can cause myelosuppression and hepatotoxicity.
- **Side effects**: nausea, vomiting, diarrhea, pancreatitis, anemia, leukopenia, thrombocytopenia, and weight loss.
- Usual dose: 1-2 mg/kg/d

Mycophenolate Mofetil (MMF) Mycophenolic Acid (MPA)

- Inhibit de novo purine nucleotide synthesis by abrogation of inosine monophosphate dehydrogenase and production of guanosine nucleotides.
- Blocks DNA replication in T & B lymphocytes which are unable to use alternate salvage pathways.
- Liver dysfunction increase half life by decreasing conjugation; albumin levels change pharmacokinetics.
- More than 50% on transplant programs use them.
- Dose reduction and withdrawal are needed in 24-57%.

MMF & MPA
Toxicity & Dose

- Nausea, vomiting, abdominal pain, diarrhea, anemia, leukopenia, thrombocytopenia, hypercholesterolemia, hypokalemia, tremor, hypertension, edema.
- MMF: 2-3 g/day, divide q 12h
- MPA: 720-1440 mg/d divided q 12h.

Drug-Drug Interaction Azathioprine & Mycophenolate

Increases AZA	**Increases MMF**	**Decreases AZA & MMF**
Allopurinol Methotrexate ACE inhibitors	Probenecid Tacrolimus	Cholestiramine Antacids Iron preparations

Triple Therapy
Prednisone + CNI + MMF

- Improves patient & graft survival in HCV & Non-HCV.
- Lower ACR rate in HCV & Non-HCV
- Less renal toxicity with lower level of CNI.
- Does not increase risk of infection nor malignancy.

Rapamycin

- Macrocyclic triene antibiotic with immunosuppressive, antitumor & antifungal properties
- Binds to immunophilin FKBP12 but has different action than TAC: blocks cell-cycle progression at the “G1 – S phase” junction.
- Increase risk of Hepatic Artery Thrombosis: “The safety and efficacy of Sirolimus…has not been established in liver transplant patients, and therefore such use is not recommended”.

Rapamycin Toxicity & Dose

- Anemia, hypercholesterolemia, hypertrigliceridemia, hyperlipidemia, leukopenia, thrombocytopenia, interstitial lung disease, peripheral edema, wound dehiscence, lymphocele, oral ulcers.
- Dose: 2 mg/d, adjusted to maintain trough level of 4-10 ng/mL.

Drugs that Increase Rapamycin Concentration

Calcium Channel Blockers	**Antifungals**	**Macrolide antibiotics**	**Pro-kinetics**	**Miscellaneous**
Diltiazem Nicardipine Nifedipine Verapamil	Fluconazole Itraconazole Ketoconazole Voriconazole Clotrimazole	Clarithromycin Erythromycin Troleandomycin Azithromycin Telithromycin	Cisapride Metoclo_pramide	Amiodarone Cimetidine Omeprazole Methyl-prednisolone Protease inhibitors **CyA**

Drugs that Decrease Sirolimus Concentration

Anticonvulsants	**Antibiotics**	**Herbal Preparations**
Carbamazepine Phenobarbital Phenytoin Fosphenytoin	Rifabutin Rifampin Rifapentin	St. John’s Wort

Special Conditions to use Rapamycin

HCC	Anti-tumor Effect
HCV & PSC	Anti-fibrotic Effect
Renal Insufficiency	Spares CNI

Antithymocyte Globulin (ATG)

- ATGAM (equine) and Thymoglobulin (rabbit)
- Polyclonal Ab against T-cells epitopes (CD2, CD3, CD4, CD8, CD28, & T-cell receptor), NK cells epitopes (CD16), and macrophages.
- Cause T-cell depletion by: apoptosis, antibody mediated cytolysis, and internalization of cell surface receptors.
- First dose can cause “cytokine release S”: fever, chills, tachycardia, chest pain, bronchospasm, GI disturbances, blood pressure changes. Steroids + Benadryl + acetaminophen helps.
- Used in 6% of US transplant programs.
- Dose: 1.5-5 mg/kg/d over 4-6 h infusion, for 3-5 days.

Muramonab-CD3 (OKT3)

- Murine Ab against T-cell CD3 antigen; inactivates T-cell receptor.
- Cytokine release syndrome is very common 1-3 h after first dose. Sometimes life-threatening with pulmonary edema and shock.
- Re-exposure to OKT3 may decrease efficacy.
- Dose: 5 mg IV q day x 10-14 days for steroid resistant rejection.

IL-2 receptor antibodies Basiliximab & Daclizumab

- Basiliximab (Simulect) is chimeric, Daclizumab (Zenapax) is humanized;
- Bind to IL-2R alpha-chain present in activated T-lymphocytes. Causes competitive antagonism of IL-2 induced T-cell proliferation.
- Effect up to **3 weeks with Basiliximab**, and **10 weeks with Daclizumab**.
- Side effects are mild.
- Dose:
  - a) Basiliximab: 20 mg IV pre-op + 20 mg 4 d later.
  - b) Daclizumab: 1 mg/kg every 14 days x 5 doses.

Steroid Avoidance

- **Reason**: Minimize osteoporosis, hyperglycemia, hypertension, hyperlipidemia, infections, Cushingoid features, and HCV recurrence.
- **TAC+MMF+Thymoglobulin vs. TAC+MMF+Steroids**:
  - F/U 1.5 y, graft survival 89% in both, rejection (20 vs 32%, $p<0.05$), recurrent HCV (50 vs 71%, p=ns)
- **TAC+daclizumab vs TAC+Steroids**:
  - F/U 3 months, daclizumab group had less steroid resistant rejection, DM, and CMV infection
- ***Larger randomized studies with longer F/U are needed.***

Renal Sparing Protocols

- Up to 21% of LTx patients develop CRF within 5 years.
- 18% of patients have ***severe*** renal dysfunction after 13 years.
- Adding MMF and reducing dose of calcineurin inhibitor (CNI) can improve GFR by 15% in 50% of patients; if CNI is D/C, rejection risk is increased.

Conversion from CNI to Sirolimus

- 28 patients with creatinine > 1.8 mg/dL were converted; mean time= 2y post-LTx.
- Dose: 2 mg/d, titrated to 4-10 ng/mL.
- 14 (50%) had improvement in GFR;
  7 progressed to ESRD, and
  6 did not tolerate the change.
- Large randomized trials are ongoing to evaluate proper time to change.

Effect of Steroid-Withdrawal after 3 months post OLTx

	Steroids	No-Steroids	P-value
Survival	82 %	83 %	NS
Hypertension	**58 %**	**15 %**	**0.0002**
Diabetes	**25 %**	**6 %**	**0.007**
Infections	**17 %**	**2 %**	**0.05**
Recurrent HCV	17 %	21 %	NS
Bone Disease	**9 %**	**0 %**	**0.05**
Acute Rejection	8 %	4 %	
Chronic Rejection	1 %	2 %	
Mean Cholesterol	**253 mg/dL**	**183 mg/dL**	**0.001**