**IMMUNOSUPPRESSIVE THERAPY**

**Overview**

**Two types of immune responses to allografts:**
- **Cellular response:** foreign antigen recognition → activate antigen-specific lymphocytes (T-cells)
  - Key mediator: T-cells
- **Humoral response:** foreign antigen recognition → antibody formation by immune system (B-cells)

**Mechanisms of immunosuppression**
- Depletion of lymphocytes
- Diversion of lymphocyte traffic
- Blocking of lymphocyte response

**Stages of immunosuppression**

**Desensitization**

**Overview**

<table>
<thead>
<tr>
<th>Who</th>
<th>What</th>
<th>When</th>
<th>Why</th>
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<tbody>
<tr>
<td>Recipients with antibodies against their living donor (HLA or ABO incompatible)</td>
<td>Immunosuppressive therapy to lower recipient’s level of antibodies against the donor to allow for transplant</td>
<td>1 to 2 weeks prior to transplant</td>
<td>Improved outcomes compared to waiting for cadaveric donor; gives highly sensitized recipients a chance</td>
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**Determining recipient’s need for desensitization**

- **Measuring ABO incompatibility**
  - Naturally occurring antibodies against donor’s blood type antigen (e.g. type A, type B, type AB, type O)
  - Mix recipient serum with antibodies + donor’s blood type antigen → serial dilutions to find ABO titer
  - 1:4 titer is needed to proceed with transplant
- **Measuring HLA incompatibility**
  - Non-naturally occurring antibodies against HLA that the recipient has either due to pregnancy, blood transfusion, or a previous transplant
  - 3 methods of measuring antibodies to HLA
    - **PRA:** panel reactive antibody
      - Tests recipient vs. population (i.e. probability of incompatibility)
      - ↑PRA% = ↑reactivity to new organ = ↑risk of antibody-mediated rejection
    - **Crossmatch**
      - Tests recipient vs. donor
      - Two methods to test: standard crossmatch & flow crossmatch
        - Standard crossmatch: cytotoxicity test → damage signifies (+) crossmatch
        - Flow crossmatch: flow cytometer → quantified antibody response but can’t see which antibody it is reacting to (non-specific)
      - If patient has positive crossmatch, they are HLA incompatible
    - **DSA:** donor-specific antibody
      - Tests recipient vs. donor
      - Determines exactly which antibody it is reacting to, the most sophisticated test
      - Method: beads with chemically attached HLA antigens + recipient serum + fluorescent-labeled anti-human IgG → processed through Luminex Analyzer

**Desensitization strategies**

- Mechanism: removal or reduction of donor-specific antibodies

**PRIBES**
• **Plasmapheresis**
  - Mechanical removal of antibodies from patient’s blood
  - Method: remove blood from patient → remove plasma (with antibodies) from blood and discard → replace plasma with albumin or fresh frozen plasma

• **IVIG** (intravenous immunoglobulin)
  - Functions: replace essential antibodies removed with plasmapheresis, helps downregulate production of antibodies by inhibiting complement system
  - SE: infusion-related reactions, flushing, fever, chills, arthralgias, dyspnea

• **Rituximab** (Rituxin)
  - Chimeric mouse monoclonal antibody, *depleting agent*
  - MOA: binds to CD20 on B-cells → apoptosis → ↓ antibody production
  - Does not affect already existing antibodies or plasma cells
  - SE: hypotension, bronchospasm, mucocutaneous reactions
  - May premedicate (APAP, Benadryl) or treat (hydrocortisone, epinephrine)

• **Bortezomib** (Velcade)
  - Proteasome inhibitor, *depleting agent*
  - MOA: (→)proteasome complex involved in protein processing (e.g. plasma cells) → apoptosis of plasma cell
  - Does not affect already existing antibodies or plasma cells
  - SE: peripheral neuropathy, neutropenia, thrombocytopenia

• **Eculizumab** (Soliris)
  - Humanized monoclonal antibody specific for complement system
  - MOA: (→)membrane attack complex formation → interrupts terminal step of complement cascade
  - SE: headache, back pain, URI symptoms, nausea

• **Splenectomy**
  - The spleen concentrates B-cells around its blood vessels in order to fight infection
  - MOA: surgically remove spleen → deplete antibodies and memory cells
  - For ABO incompatibility
  - Important for patient to get vaccinated: pneumococcal, meningococcal, hemophilus influenza B

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**Induction**

**Overview**

- Acute & potent immunosuppression in the peri-operative period generally for high risk patients to ↓ risk of rejection
- Purpose: suppress T-cells to encourage allograft acceptance + bridge to maintenance therapy
- Primarily for high risk patients: African Americans (stronger immune systems), re-transplants, & highly sensitized patients (HLA incompatibility due to pregnancy, blood transfusions, or re-transplants)

**Agents for induction**

-- “STAB”

  - **Steroids**: methylprednisolone taper, oral prednisone
    - Corticosteroids, *non-depleting agents*
    - MOA: blocks cytokine gene expression → anti-inflammatory effect + redistributes lymphocytes
    - High dose IV methylpred: 250mg – 1000mg
    - Pharmacokinetics: t½ 2-3 hrs, duration of action 24 hrs
    - Short-term SE: impaired glucose tolerance, ↑ bp, ↑ appetite, impaired wound healing, mood disturbances
    - Long-term SE: osteoporosis, diabetes, acne, cataracts, infection, ↑ weight, hyperlipidemia, steroid-dependence
    - Always used in induction, even in steroid-free programs

  - **Thymoglobulin or Atgam**
    - Polyclonal antibody, *depleting agents*
    - Derived IgG antibodies from rabbits (Thymoglobulin) or horses (Atgam)
    - MOA: polyspecific binding of antibodies to immunocompetent T-cells using surface antigens → rapid lymphopenia
    - Thymoglobulin preferred over Atgam unless rabbit allergy: longer and ↑ T-cell depletion, ↑ affinity to T-cells
    - Thymoglobulin dose: 1.5 mg/kg IV for 3-7 days
    - SE: leukopenia, thrombocytopenia, anemia, lymphoproliferative disease
    - Need to try to ↓ response to foreign protein (e.g. cytokine release syndrome, serum sickness)
      - Premedicate: APAP 325-1000mg, Benadryl 25-50mg, or methylprednisolone
      - Prolong infusion rate (~ 6 hours) and/or infuse via central access
    - Monitor for efficacy (ALC<200, CD3<50) and toxicity (WBC<5, platelets<100)

  - **Basiliximab** (IL-2 RA)
- **Chimeric monoclonal antibody, non-depleting agent**
  - MOA: antibody directed against CD25 on activated T-cell surface → halts T-cell proliferation
  - SE: generally well tolerated

- **Alemtuzumab**
  - Humanized monoclonal antibody, depleting agent
  - MOA: antibody directed against CD52 (on T-cells, B-cells, monocytes, macrophages, NK cells) → cell surface binding → cellular-mediated lysis
  - Dose: 20mg IVPB or 0.3 mg/kg/day x 2 doses
  - Adverse effects
    - Infusion reactions: fevers, chills, rash, N/V/D, dyspnea → limit with APAP, Benadryl, or methylpred
    - Hypotension → limit with fluids ± vasopressors
    - Hematologic effects: anemia, neutropenia, thrombocytopenia → limit with erythropoietin, filgrastim, transfusion (in this order)
    - Infections: opportunistic infections, sepsis

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**Maintenance immunosuppression**

Drugs administered post-transplant for lifelong immunosuppression to prevent allograft rejection

**Calcineurin inhibitors**

- Cornerstone of immunosuppressive therapy
- MOA: blocks IL-2 signal production
- Toxicities: nephrotoxicity, neurotoxicity, electrolyte disturbances, DM, HTN
  - Nephrotoxicity: both acute & chronic
    - Additive nephrotoxicity with NSAIDs, aminoglycosides, amphotericin B
    - Nephroprotective effects: ACE-I, ARB, CCB (dihydropyridine)
  - Diabetes: tacrolimus >> cyclosporine
- Drug interactions: CYP3A4 metabolized
  - CYP3A4 inducers: phenytoin, carbamazepine, rifampin
  - CYP3A4 inhibitors: azoles, macrolides, CCB (non-dihydropyridines), antiretrovirals
  - P-gp substrates: digoxin, statins, colchicine
    - Diarrhea ↓ P-gp expression → ↑ CNI levels

- **Tacrolimus** (Prograf)
  - MOA: binds to FKBP-12 → (–)calcineurin → (–)IL-2 synthesis → prevents T-cell proliferation
  - Dose: PO 0.05-1 mg/kg/dose q12h, IV 50-100 mcg/kg/day as continuous infusion
    - Dose adjustments: based on trough
  - SE: alopecia, tremors/falls

- **Cyclosporine**
  - Two formulations:
    - Sandimmune: O/W cyclosporine emulsion
    - Neoral: microemulsion cyclosporine
  - MOA: binds to cyclophilin → (–)calcineurin → (–)IL-2 synthesis → prevents T-cell proliferation
  - Dose: PO 3-5 mg/kg/dose q12hr, IV 50% of PO dose over 2-6hr continuous infusion
    - Dose adjustments: based on troughs or 2hr level
  - SE: hirsutism, gingival hyperplasia, hyperuricemia, hyperlipidemia
  - Role in therapy: kids > 5 y/o, EBV naïve

**Antimetabolites**

- **Azathioprine**
  - Prodrug of 6-mercaptopurine
  - MOA: nucleoside analog that inhibits purine synthesis
  - Dose: PO 1-3 mg/kg/day, IV equivalent oral dose
    - Dose adjustment: based on SE, levels not monitored

**Steroids**

- **Methylprednisolone**

**mTOR inhibitors**

- **Sirolimus** (Rapamune)
- **Everolimus** (Zortress)

**Belatacept** (Nulojix)

Most common maintenance regimen: tacrolimus + MPA + Steroids
Drug interactions: allopurinol → blocks xanthine oxidase → ↑6 mercaptopurine → severe infection
○ SE: hematologic (leukopenia, anemia, thrombocytopenia), pancreatitis, hepatotoxicity, squamous skin cell cancer
○ Place in therapy: too many SE, no longer used much

### Mycophenolate

- Prodrug of mycophenolic acid
- MOA: (–) IMPDH → (–) de novo synthesis of purines
- Two formulations:
  - Mycophenolate mofetil (Cellcept)
  - Enteric-coated mycophenolate sodium (Myfortic)
- MMF dose: PO 1000–1500mg bid, IV 1000mg bid over 2hr
- Dose adjustment: based on SE, too difficult to monitor levels
- Metabolic pathway: enterohepatic recirculation → stays in system longer
- Cyclosporine inhibits this recirculation, therefore combination with tacrolimus more effective
- SE: GI upset (N/V/D, dyspepsia, ab pain; more than azathioprine), hematologic (less than azathioprine)
- EC formulation helps ameliorate upper GI effects, better tolerated
- Role in therapy: preferred over azathioprine because ↓ acute rejection, ↑ graft survival, ↓ hematologic effects

### mTOR inhibitors

- **Agents:** Sirolimus, Everolimus
  - mTOR = mammalian target of rapamycin
- **mTORi MOA:** binds to FKBP-12 → (–)mTOR activity → ↓ phosphorylation of proteins → ↓ translation & ↓ protein synthesis → ↓ proliferation of lymphocytes
- Overall blocks IL-2 signal transduction
- Sirolimus dose: PO 2mg or 0.1mg/kg, no IV formulation
- Dose adjustments: based on levels or toxicities
- Drug interactions: CYP3A4 metabolism & P-gp substrate (admin 4hrs after cyclosporine)
- SE: profound hematologic (anemia, thrombocytopenia), proteinuria, nephrotoxicity, hypertriglyceridemia, mouth ulcers, additive nephrotoxicity with calcineurin inhibitors, impaired wound healing, interstitial pneumonitis
- Place in therapy: third line, use if can’t tolerate all others
- Sirolimus: also used in cardiology: anti-VEGF properties → (–) smooth muscle proliferation
- May have benefits in ↓ malignancies

### Corticosteroids

- **Agent:** prednisone low dose (5-15mg qd)
- Many adverse effects → 50% of US transplant centers use steroid free regimens
- Short term: impaired glucose tolerance, ↑ bp, ↑ appetite, impaired wound healing, mood disturbances
- Long term: osteoporosis, diabetes, acne, cataracts, infection, weight gain, hyperlipidemia, dependence
- Place in therapy: patients failing steroid-free regimen, very high risk sensitized patients with preformed antibodies, no induction (3 drug regimen)

### Belatacept

- Only one in its class: selective co-stimulation signal 2 blocker
- MOA: (–)APC’s CD80/86 from binding to T-cell CD28 receptor → (–)signal 2 → (–)cytokine production → (+)T-cell anergy & apoptosis → (–)proliferation
- Administration: patient has to come into office for 30min IV infusion once a month
- SE: myelosuppression, post-transplant lymphoproliferative disorder (if EBV neg), GI upset, neuropathy, infusion-related SE
- Contraindication: patients EBV naïve (e.g. young children) → PTLD
- Place in therapy: only used for kidney transplants in conjunction with mycophenolate and steroids

### Overview of maintenance therapy toxicities

<table>
<thead>
<tr>
<th>Category</th>
<th>Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcineurin inhibitors</td>
<td>Nephrotoxicity, neurotoxicity, hypertension, diabetes, hyperlipidemia,</td>
</tr>
<tr>
<td></td>
<td>electrolyte disorders, hirsutism/alopecia, GI toxicity</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>GI toxicity, cytopenia</td>
</tr>
<tr>
<td>mTOR inhibitors</td>
<td>Hyperlipidemia, anemia, edema, mouth ulcers</td>
</tr>
<tr>
<td>Steroids</td>
<td>Osteoporosis, diabetes, weight gain, body changes, glaucoma, wound healing</td>
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Immunosuppressive Pharmacology | Optimization

>> Why it matters

Recipient survival: live donor > deceased donor

Current problems: chronic rejection, nephrotoxicity from treatment, & death with functioning graft (due to CVD, malignancy, infections)

CVD: CNI & steroids > mTORi >>> mycophenolate & belatacept (n/a)

Immunosuppressive therapy | Choosing the right regimen

>> Three questions to think about

- Patient’s risk for rejection?
  - Induction vs. no induction
    - 3 groups who should get induction
      - 1. High risk for rejection
        - High risk: immunogenicity of organ transplanted, African Americans, highly sensitized patients with preformed antibodies
        - Should use depleting agents (e.g. Thymoglobulin)
      - 2. Experimental protocols
        - Steroid-free protocols (e.g. UICMC)
        - Calcineurin avoidance protocols
      - 3. High risk for ATN resulting in delayed graft function
        - ATN: acute tubular necrosis, due to ischemic damage
        - High risk: donor >50y/o, donor <12y/o, recipient >55y/o, cold ischemic time >24hr, donor SCr >1.8mg/dL, DCD donor (cardiac death)
        - Just an estimated risk evaluation performed with limited info available; not enough time to see what the kidney actually looks like
        - May warrant a delay in initiating calcineurin inhibitors
    - Depleting vs. non-depleting agent
  - Which calcineurin inhibitor?
    - Cyclosporine vs. tacrolimus vs. none
      - Potency: tacrolimus > cyclosporine
      - DM risk: tacrolimus > cyclosporine
      - Patient age: if <5 y/o, use cyclosporine (EBV naïve)
      - Cyclosporine SE: hirsutism, gingival hyperplasia, hyperuricemia, hyperlipidemia
      - Tacrolimus SE: alopecia, tremors, falls, diabetes
    - Delayed CNI initiation
      - If high risk for ATN
    - CNI avoidance
      - Benefits: ↓long term renal damage, ↓SE (DM, HTN, electrolyte abnormalities)
      - Patients with ATN are at risk for delayed graft function
        - May need induction to avoid additive CNI toxicity
      - Disadvantages: delaying CNI may ↑rejection risk, SE of other meds are not very favorable either
      - Alternative options: basiliximab (induction) and belatacept (maintenance) → using these as adjuncts or alternatives can help ↓CNI toxicities
  - Adjunct agent combination?
    - Mycophenolate vs. mTORi
      - Mycophenolate SE: GI problems, leukopenia, viral infections
      - Sirolimus SE: impaired wound healing (don’t use in fresh surgery patients), lipids, nephrotoxicity (especially in combination with CNI), proteinuria
      - Liver vs. kidney: if liver transplant, mycophenolate may be preferable
      - Hepatocellular cancer: sirolimus has antitumor properties
    - Steroid minimization or avoidance
      - Both short term and long term SE affect outcome of transplanted graft
      - Benefits: ↓PTDM, ↓weight gain, ↓hyperlipidemia
      - Risks: ↑doses of tacrolimus/cyclosporine, ↑potent induction, ↑SE of other meds
      - Not eligible for steroid-free program: on steroids before transplant, ABO-incompatible, positive crossmatch, re-transplant, recurrent/early rejection episodes
Current UIHSS practice: kidney transplants
- **Induction:** low risk gets basiliximab and methylprednisolone, high risk gets thymoglobulin and methylpred
  - If induction with methylpred only, then patient will need lifelong steroid maintenance
- **Maintenance:** everyone gets tacrolimus except low risk non-DM Hispanics

### Rejection

#### Classification of rejection based on time to rejection

- **[minutes]** Hyperacute
- **[days to weeks]** Acute
- **[>3 months]** Late acute
- **[months to years]** "Chronic"

#### Pathophysiological classification
- **Cellular rejection (T-cell)**
  - Major players: APCs (macrophages, B-cells, etc.), antigens, MHC, T-cells
  - Mechanism: T-cells secrete cytokines → inflammation → apoptosis, interstitial fibrosis, and other graft damage
  - Upon biopsy: inflammation, tubulitis
- **Antibody-mediated rejection**
  - Major players: cytokines, chemokines, chemoattractants
  - Mechanism: complement activation → antibodies target MHC antigen on endothelium of donor peritubular & glomerular capillaries → inflammation → rapid graft dysfunction
  - Upon biopsy: cell adhesion to endothelium, C4d staining, glomerulitis

#### Histological classification | Banff Criteria
- **T-cell mediated rejection**
  - Acute T-cell mediated rejection: order of severity
    - [least severe] IA, IB, IIA, IIB, III [most severe]
  - Chronic T-cell mediated rejection: chronic allograft arteriopathy
- **Antibody-mediated changes**
  - C4d + circulating DSA + no evidence for rejection
  - Acute AMR: C4d + circulation DSA + acute tissue injury
  - Chronic AMR: C4d + circulating DSA + chronic tissue injury

#### Additional classifications of rejection
- Based on response to treatment: steroid-resistant rejection
- Based on renal damage: acute rejection (biopsy proven + ↑SCR) vs. subclinical rejection (biopsy proven w/o ↑SCR)

#### Risk factors for rejection: HLA incompatibility, ABO incompatibility, African American race, longer cold ischemia time

#### Signs & symptoms of rejection: fever (>38°C), graft tenderness, oliguria (urine output <500 mL/day), hypertension, ↑SCR (>25%↑)

#### Differential diagnosis

- **Pre-renal**
  - Dehydration
  - Hypotension
  - Renal artery narrowing

- **Renal**
  - Nephrotoxicity (e.g. drugs)
  - Rejection

- **Post-renal**
  - Obstruction

#### Diagnostic criteria: biopsy is the gold standard, though lab values give a clue (e.g. ↑SCR)
Treatment

- Treating acute cellular rejection
  - Corticosteroids
    - MOA: ↓inflammation, ↓neutrophil adherence, depletes APCs, ↓T-cell proliferation, ↓prostaglandins
    - Role in therapy: 1st line agent for ACR, though often inadequate alone when severe rejection or AMR
  - Thymoglobulin
    - MOA: depletes T-cells, (-) co-stimulation of T-cells
    - Role in therapy: 2nd line due SE, used in combination with steroids when more severe rejection
- Treating antibody-mediated rejection: plasmapharesis, IVIG, corticosteroids (ineffective as monotherapy), rituximab, bortezomib, eculizumab

Post-rejection management: stress medication adherence, optimize maintenance regimen, infection prophylaxis (Bactrim SS, valganciclovir)