Immunopharmacology

PHARMACOLOGY 3

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Immunosuppressants

Immunosuppressants are used:

1. To prevent rejection in patients receiving organ transplants
2. In various autoimmune diseases (e.g, rheumatoid arthritis, multiple sclerosis, Crohn disease, and ulcerative colitis)

Immune system activation cascade

The immune activation cascade can be described as a **three-signal model**:

- **Signal 1** constitutes T-cell triggering at the **CD3 receptor** complex by an antigen on the surface of an antigen-presenting cell (APC).

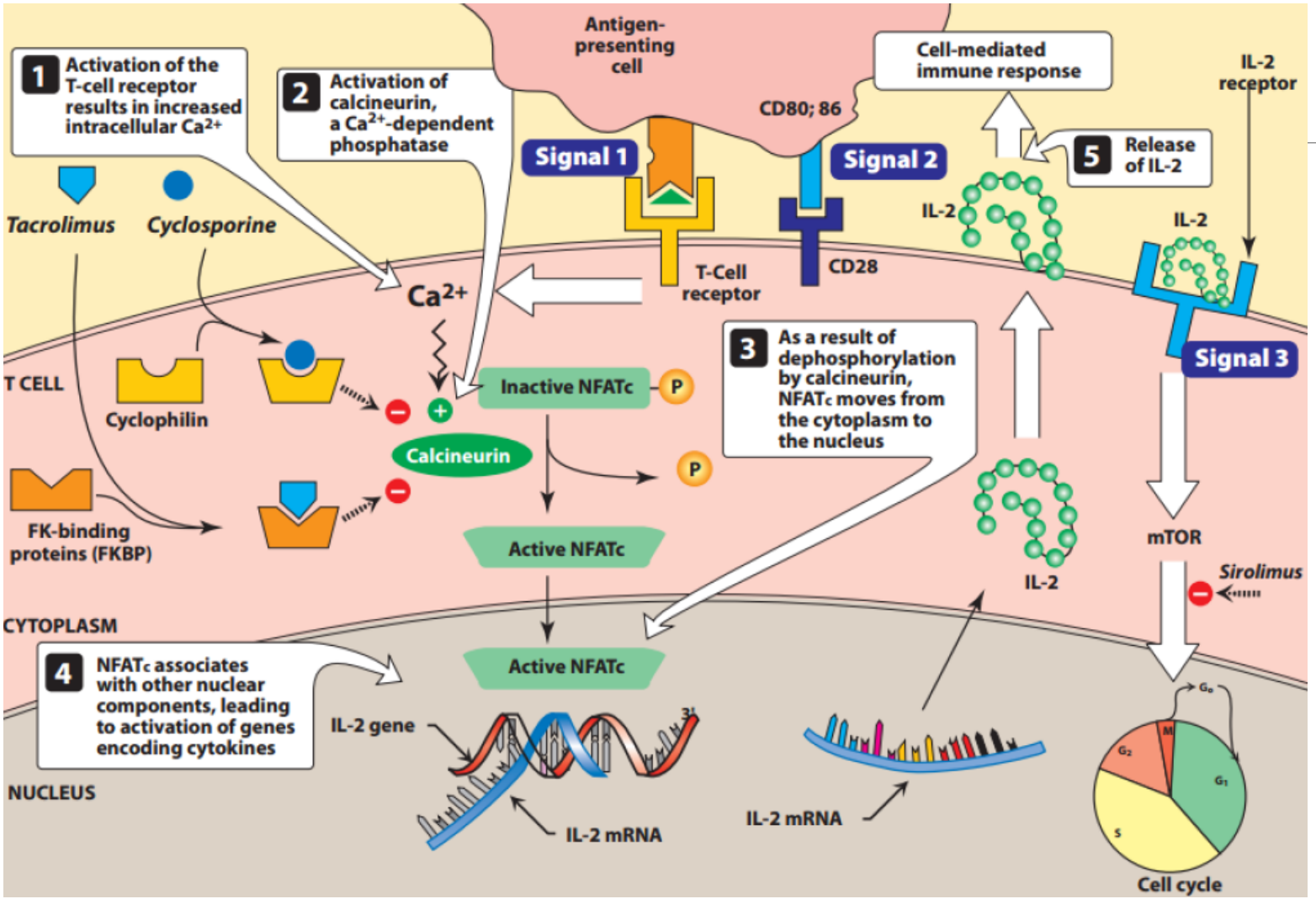
Signal 1 alone is insufficient for T-cell activation and requires signal 2.

- **Signal 2** (also referred to as costimulation) occurs when **CD80 and CD86** on the surface of APCs engage CD28 on T cells.

Both Signals 1 and 2 activate several intracellular signal transduction pathways, one of which is the calcium-calcineurin pathway.

Immune system activation cascade

- These pathways trigger the production of cytokines such as interleukin **(IL)-2**, and T-cell dependent activation of B lymphocytes.
- IL-2 then binds to **CD25** (also known as the **IL-2 receptor**) on the surface of other T cells to activate mammalian target of *rapamycin* (mTOR), providing **Signal 3**, the stimulus for T-cell proliferation.


1 Activation of the T-cell receptor results in increased intracellular Ca2+
2 Activation of calcineurin, a Ca2+-dependent phosphatase
Antigen-presenting cell
CD80; 86
Signal 1
Signal 2
Cell-mediated immune response
IL-2 receptor
5 Release of IL-2
IL-2
IL-2
Tacrolimus
Cyclosporine
T-Cell receptor
CD28
Ca2+
Signal 3
T CELL
Cyclophilin
Inactive NFATc
P
3 As a result of dephosphorylation by calcineurin, NFATc moves from the cytoplasm to the nucleus
Calcineurin
P
FK-binding proteins (FKBP)
Active NFATc
IL-2
mTOR
Sirolimus
CYTOPLASM
4 NFATc associates with other nuclear components, leading to activation of genes encoding cytokines
Active NFATc
IL-2 gene
IL-2 mRNA
IL-2 mRNA
NUCLEUS
Cell cycle

Immunosuppressive agents

- Immunosuppressive drugs can be categorized according to their mechanisms of action:

1. Drugs interfere with cytokine production or action;
2. Drugs disrupt cell metabolism, preventing lymphocyte proliferation;
3. Mono- and polyclonal antibodies block T-cell surface molecules.

- Because of their severe toxicities when used as monotherapy, a combination of immunosuppressive agents, usually at lower doses, is generally employed.

Selective inhibitors of cytokine production and function

These drugs can be further divided into three main classes:

1. Calcineurin inhibitors (**cyclosporine** and **tacrolimus**)
2. Co-stimulation blockers (**belatacept**)
3. mTOR inhibitors (**sirolimus** and **everolimus**).

Cyclosporine

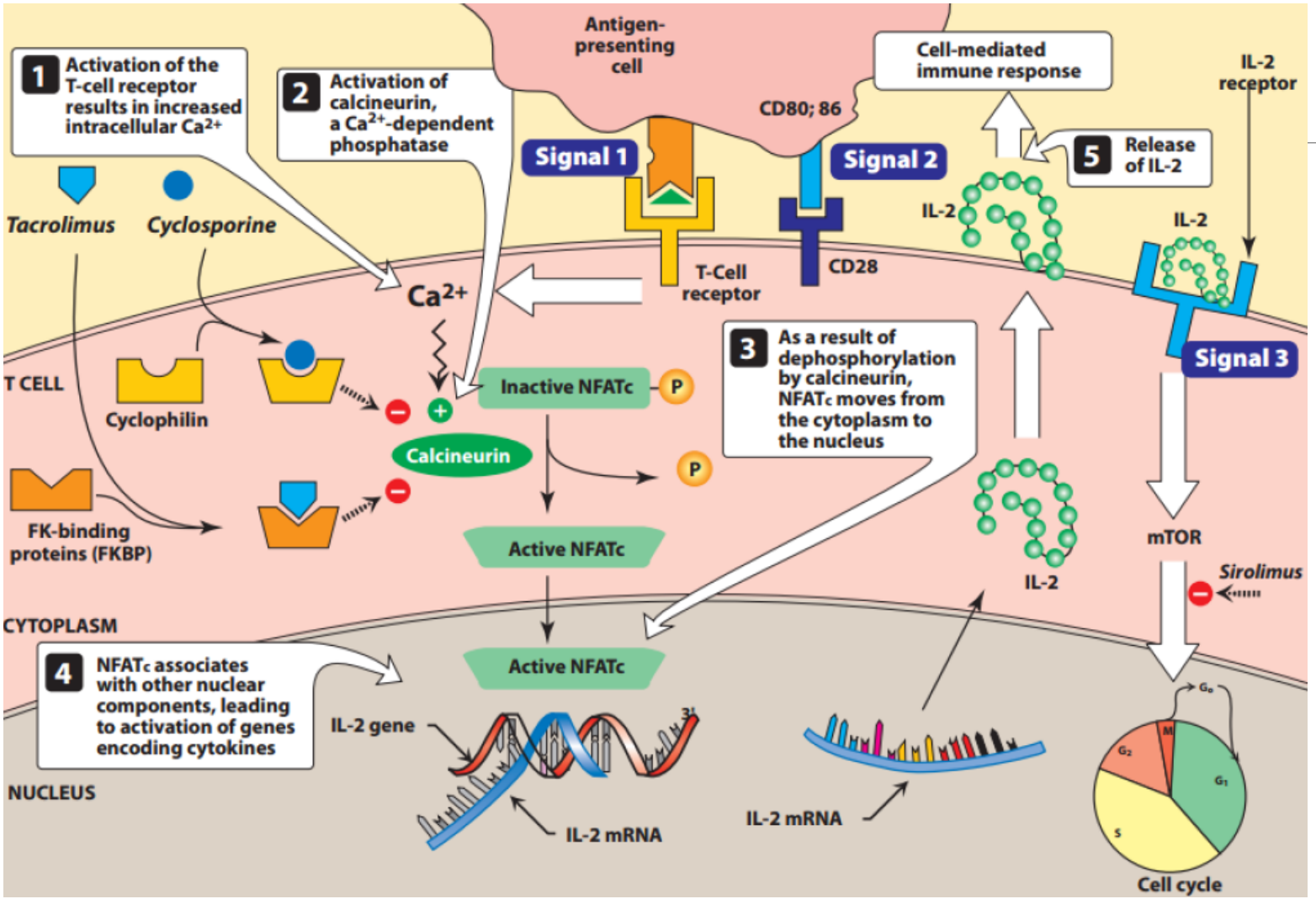
Used for:

1. Prevention of graft-versus-host disease in hematopoietic stem cell transplantation patients
2. Prevention of graft rejection in solid organ transplant patients
3. Treatment of psoriasis, rheumatoid arthritis and a variety of other autoimmune diseases

Cyclosporine

Mechanism of Action:

1. Cyclosporine binds to cyclophilin, an intracellular cytoplasmic protein found in T-cells
2. The CsA-cyclophilin complex interacts with calcineurin. The latter is responsible for dephosphorylating NFATc (**c**ytosolic **N**uclear **F**actor of **A**ctivated **T** cells).
3. CsA-cyclophilin complex inhibits the catalytic activity of calcineurin, and prevents the production of cytokines like IL-2
4. As a result, it inhibits IL-2 -induced activation of resting T-lymphocytes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Active NFATc
IL-2 gene
IL-2 mRNA
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NUCLEUS
Cell cycle

Cyclosporine

Pharmacokinetics

Cyclosporine may be given either **orally** or by intravenous **(IV) infusion**.

Oral absorption is variable due to metabolism by a CYP3A4 isoenzyme in the GI and efflux by P-glycoprotein (P-gp), which limits *cyclosporine* absorption by pumping the drug back into the gut lumen.

Excretion of the metabolites is primarily through the biliary route into the feces.

Cyclosporine

Adverse Effects:

Many of the adverse effects caused by *cyclosporine* are dose dependent. Therefore, it is important to monitor blood levels of the drug.

1. **Nephrotoxiciy** (most common) it is critical to monitor kidney function. Reduction of the *cyclosporine* dosage can result in reversal of nephrotoxicity in most cases.
2. **Hepatotoxicity** can also occur, liver function should be periodically assessed
3. **Neurotoxicty** (headache, tremors, parasthesia, seizures, altered mental status)

Cyclosporine

Adverse Effects:

5. infections are common and may be life threatening. Viral infections due to the herpes group and cytomegalovirus (CMV) are prevalent.
6. Hypertension
7. Hyperlipidemia
8. Hyperglycemia
9. Hyperkalemia (K-sparing diuretics should be avoided in these patients)
10. Hirsutism
11. Gingival hyperplasia.

Cyclosporine

Drug interactions:

Two categories:

1. Drugs that are known to cause nephrotoxicity by themselves.
   - Ex: Aminoglycosides, NSAIDS.

2. Inhibition or induction of cyclosporine metabolism (CYP3A4 inhibitors and inducers) and P-gp inhibitors.

   (**Grapefruit** and grapefruit juice affect metabolism, increasing blood concentration of cyclosporine, thus should be avoided.)

Tacrolimus

Another calcineurin inhibitor.

This drug is preferred over cyclosporine because of its increased potency, decreased episodes of rejection.

Used for:

- Prevention of graft-versus-host disease in hematopoietic stem cell transplantation patients,
- Prevention of graft rejection in solid organ transplant patients (liver, heart, kidney)
- Tacrolimus ointment is currently used in the therapy of atopic dermatitis and psoriasis.

Tacrolimus

Mechanism of action:

Similar mechanism of action as cyclosporin but binds with a different immunophilin called **FKBP**

Tacrolimus binds to FK-binding protein (FKPB), an intracellular cytoplasmic protein found in T-cells.

The tacrolimus-FKPB complex interacts with calcineurin, inhibits the catalytic activity of calcineurin, and blocks the production of intermediaries involved with the expression of genes regulating the production of cytokines (IL-2)

Tacrolimus

Pharmacokinetics:

- Tacrolimus may be administered **orally** or **IV**.
- The oral route is preferable, but, as with cyclosporine, oral absorption of tacrolimus is incomplete and variable, requiring tailoring of doses.
- Tacrolimus is subject to gut metabolism by CYP3A4/5 isoenzymes and is a substrate for P-gp. Together, both of these mechanisms limit the oral bioavailability of tacrolimus.
  - grapefruit should be avoided.
  - Absorption is decreased if the drug is taken with high-fat or high-carbohydrate meals.

Tacrolimus

Adverse Effects:

- **Nephrotoxicity and neurotoxicity** (tremor, seizures, and hallucinations) tend to be more severe in patients who are treated with tacrolimus than in patients treated with cyclosporine, therapeutic drug monitoring is required and careful dose adjustment can minimize this problem .
- Development of post-transplant insulin-dependent diabetes mellitus is a problem.

Tacrolimus

Adverse Effects:

- Other toxicities are similar to cyclosporine, except that tacrolimus does not cause hirsutism or gingival hyperplasia, but it can cause alopecia.
- Compared with cyclosporine, tacrolimus has a lower incidence of cardiovascular toxicities, such as hypertension and hyperlipidemia, both of which are common comorbidities in kidney transplant recipients.
- Drug interactions are similar to cyclosporine.

Co-stimulation blocker

Belatacept, a second-generation costimulation blocker, is a recombinant fusion protein that targets signal 2 in the immune activation cascade.

It is used for long-term maintenance immunosuppressive therapy.

Mechanism of action:

Belatacept blocks CD28-mediated costimulation of T lymphocytes (signal 2) by binding to CD80 and CD86 on APCs.

This prevents the downstream stimulatory signals promoting T-cell survival, proliferation, and IL-2 production.

Co-stimulation blocker

Pharmacokinetics:

Belatacept is the first IV maintenance immunosuppressant

It is dosed in two phases. The initial high-dose phase is administered on a more frequent interval. In the maintenance phase, the dose is decreased and administered once a month.

- Monthly dosing may be beneficial in patients for whom medication compliance is an issue.

Belatacept clearance is not affected by age, sex, race, renal, or hepatic function.

Adverse effects:

Anemia, diarrhea, urinary tract infection, and edema.

Selective inhibitors of cytokine production and function

These drugs can be further divided into three main classes:

1. Calcineurin inhibitors (***cyclosporine*** and ***tacrolimus***)
2. Co-stimulation blockers (***belatacept)***
3. mTOR inhibitors (***sirolimus*** and ***everolimus***).

The mammalian target of Rapamycin (mTOR) inhibitors

The mTOR inhibitors include:

1. **Sirolimus** (previously known as rapamycin)
2. as well as its analogs such as **everolimus** and **temsirolimus**.

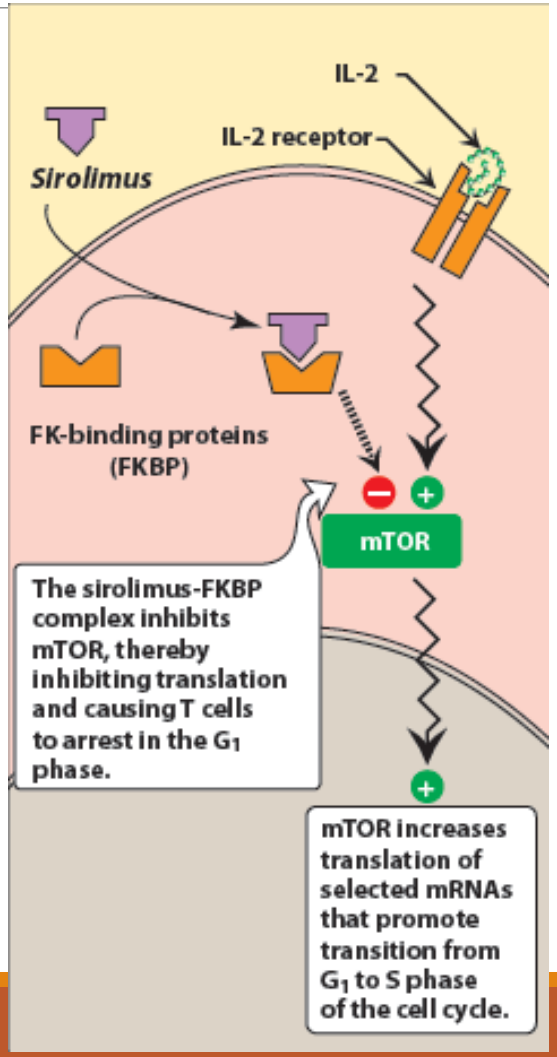
Sirolimus

Mechanism of action:

Binds to the circulating immunophilin FKBP resulting in an active complex that inhibits the kinase activity of mammalian target of rapamycin (mTOR) interfering with signal 3.

Binding of sirolimus to mTOR blocks the progression of activated T cells from the G1 to the S phase of the cell cycle and, consequently, the proliferation of these cells.

Unlike cyclosporine and tacrolimus, sirolimus does not lower IL-2 production but, rather, inhibits the cellular response to IL-2.



Sirolimus

Pharmacokinetics:

The drug is available only as **oral** preparations.

Its half-life is about 60 hours allowing for once-daily dosing.

Although it is readily absorbed, high-fat meals can decrease the drug's absorption

Metabolized by the CYP3A4 isozyme and a substrate for p-gp and interacts with the same drugs as do cyclosporine and tacrolimus

Sirolimus

Uses:

1) Sirolimus has been used effectively alone and in combination with other immunosuppressants (corticosteroids, cyclosporine, tacrolimus, and mycophenolate mofetil) to prevent rejection of solid organ allografts.

2) Sirolimus-eluting coronary stents have been shown to reduce restenosis and other adverse cardiac events in patients with severe coronary artery disease

- These benefits appear to be due to its antiproliferative effects on endothelial cells.

Sirolimus

Adverse Effects:

1. Dose dependent hypertriglyceridemia and hypercholesterolemia which may require treatment
2. Impaired wound healing
3. Profound myelosuppression (especially thrombocytopenia).

Everolimus

Everolimus (another mTOR inhibitor) is approved for use in renal transplantation.

Everolimus is rapidly absorbed, but absorption is decreased with high-fat meals.

Everolimus is a substrate of CYP3A4 and P-gp and, thus, is subject to the same drug interactions as previously mentioned.

Everolimus has adverse effects similar to *sirolimus.*

An additional adverse effect is angioedema, which may increase with concomitant use of ACEIs.

Immunosuppressive agents

- Immunosuppressive drugs can be categorized according to their mechanisms of action:

1. Drugs interfere with cytokine production or action;
2. Drugs disrupt cell metabolism, preventing lymphocyte proliferation;
3. Mono- and polyclonal antibodies block T-cell surface molecules.

- Because of their severe toxicities when used as monotherapy, a combination of immunosuppressive agents, usually at lower doses, is generally employed.

Immunosuppressive antimetabolites

- Immunosuppressive antimetabolite agents are generally used in combination with corticosteroids and the calcineurin inhibitors, *cyclosporine* and *tacrolimus.*

These include:

1. Azathioprine
2. Mycophenolate mofetil

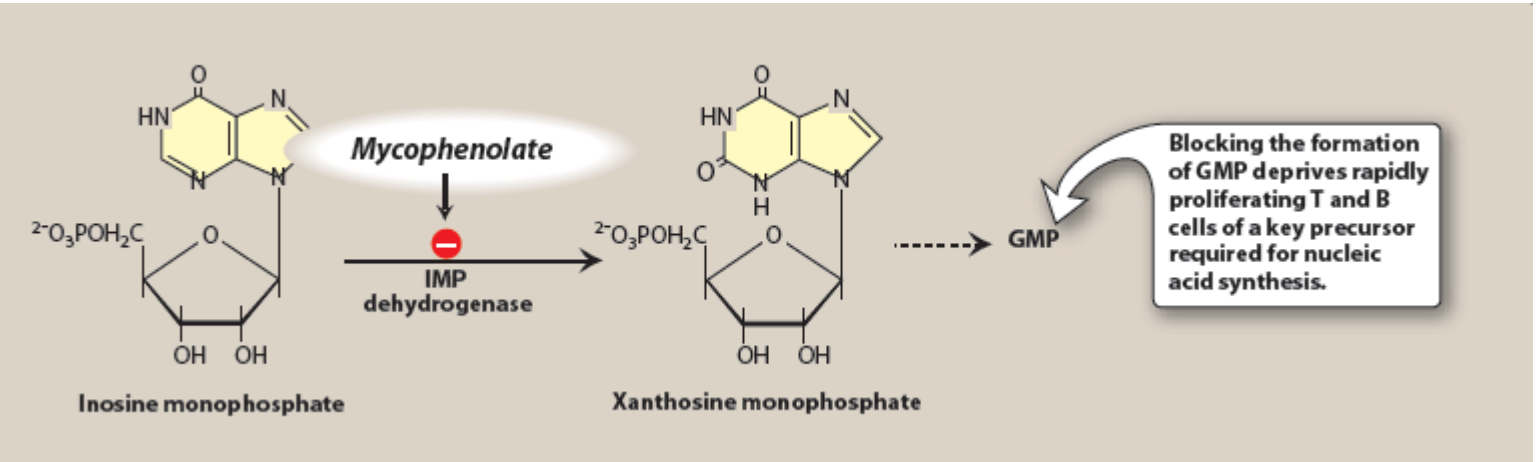
Azathioprine

- Azathioprine is a prodrug that is converted first to 6-mercaptopurine (6-MP) and then to the corresponding nucleotide **thioinosinic acid**.
  - The immunosuppressive effects of azathioprine are due to this nucleotide analog.
- lymphocytes are predominantly affected by the cytotoxic effects of azathioprine because of:
  - their rapid proliferation in the immune response
  - their dependence on the de novo synthesis of purines required for cell division
- Like 6-MP, reduce dose by 75% with allopurinol
- S/E:
- myelosuppression

Mycophenolate mofetil

- *Mycophenolate mofetil* has, for the most part, replaced *azathioprine* because of its safety and efficacy in prolonging graft survival.
- As an ester, it is rapidly hydrolyzed in the GI tract to mycophenolic acid. This is a potent **inhibitor of inosine monophosphate dehydrogenase**, which blocks the de novo formation of guanosine monophosphate. Thus, like *6-MP*, it deprives the rapidly proliferating T and B cells of a key component of nucleic acids.

Mycophenolate mofetil



Mycophenolate mofetil

- The most common adverse effects of *mycophenolate mofetil* are GI, including **diarrhea, nausea, vomiting, and abdominal pain.**
- High doses of *mycophenolate mofetil* are associated with a higher risk of **CMV infection**.
- **Drug/ drug interactions**: concomitant administration with antacids containing magnesium or aluminum, or with *cholestyramine,* can decrease absorption of the drug.

Antibodies

1. Antithymocyte globulins
2. Muromonab-CD3 (OKT3)
3. IL-2-receptor antagonists; *basiliximab and daclizumab*
4. Alemtuzumab, humanized monoclonal antibody directed against CD52

Antithymocyte globulins

- Antithymocyte globulins are polyclonal antibodies that are primarily used:

1. at the time of transplantation to prevent early allograft rejection along with other immunosuppressive agents.
2. to treat severe rejection episodes or corticosteroid-resistant acute rejection.

- **Mechanism of action:** The antibodies bind to the surface of circulating T lymphocytes. The antibody-bound cells are phagocytosed in the liver and spleen, resulting in lymphopenia and impaired T-cell responses.

Antithymocyte globulins

- Because the humoral antibody mechanism remains active, antibodies can be formed against these foreign proteins.
- Other adverse effects include:

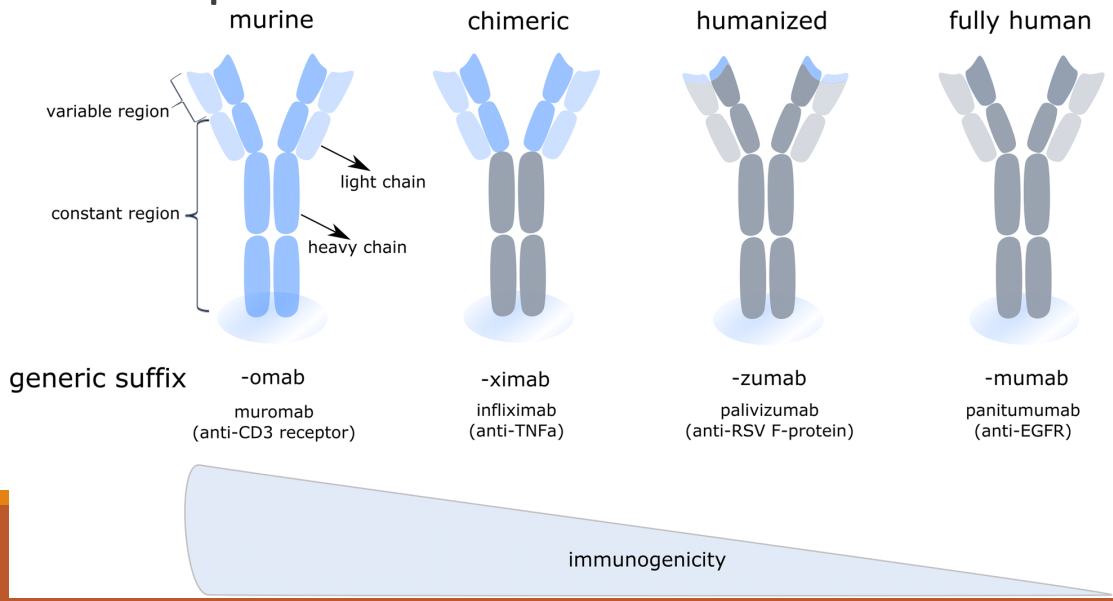
1. chills and fever
2. leukopenia and thrombocytopenia
3. infections due to CMV or other viruses
4. skin rashes.

Muromonab-CD3 (OKT3)

- *Muromonab-CD3* is a murine (mouse) monoclonal antibody directed against the glycoprotein CD3 antigen of human T cells.
- *Muromonab- CD3* was the first monoclonal antibody approved for clinical use in 1986, was used for treatment of acute rejection of renal allografts as well as for corticosteroid-resistant acute allograft rejection in cardiac and hepatic transplant patients.
- The drug has been discontinued from the market due to the availability of **newer biologic drugs with similar efficacy and fewer side effects**.

Basiliximab

- The antigenicity and short serum half-life of the murine monoclonal antibody have been averted by replacing most of the murine amino acid sequences with human ones by genetic engineering.
- *Basili**xi**mab* is said to be “chimerized” because it consists of 25% murine and 75% human protein.



Basiliximab

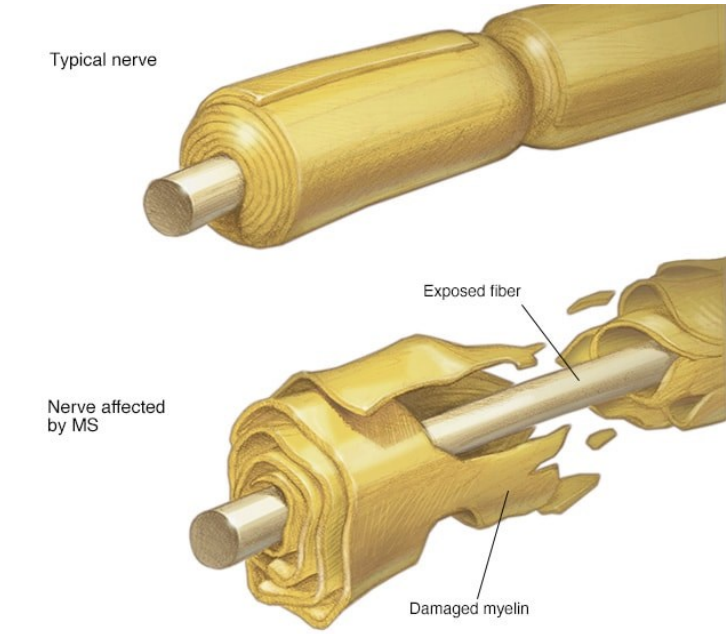
- *Basiliximab* is **an anti-CD25 antibody** that binds to IL-2 receptor on activated T cells and, thus, interferes with the proliferation of these cells.
- *Basiliximab is* indicated for prophylaxis of acute organ rejection in renal transplant patients in combination with *cyclosporine* and corticosteroids.
  - Usually, two doses of this drug are administered—the first at 2 hours prior to transplantation and the second at 4 days after the surgery.
- The drug is generally well tolerated, with **GI toxicity** as the main adverse effect.
- **Daclizumab** is a "humanized" antibody that also binds to IL-2 receptor (an anti-CD25 antibody)

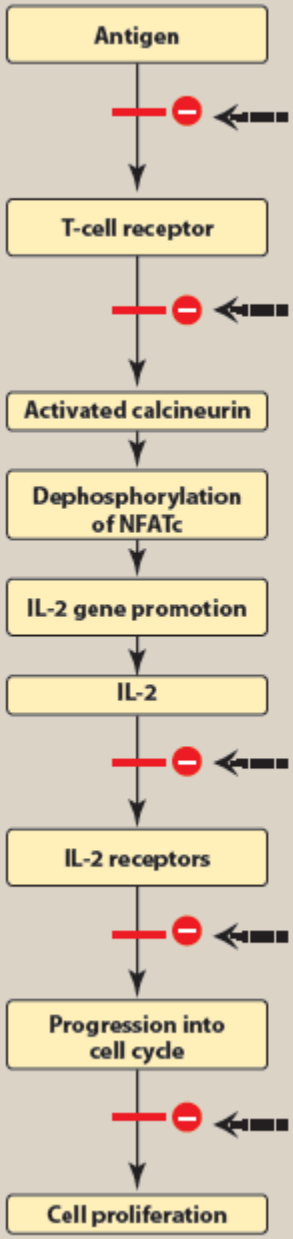
Alemtuzumab

- **Alemtuzumab** is a humanized antibody that **binds to CD52** found on normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages, and a small population of granulocytes.
- Alemtuzumab appears to deplete leukemic (and normal) cells by direct antibody-dependent lysis.
- Alemtuzumab was previously approved for the treatment of B-cell chronic lymphocytic leukemia (CLL) in patients who have been treated with alkylating agents and have failed fludarabine therapy.

Alemtuzumab

- More recently, alemtuzumab was approved for the treatment of patients diagnosed with relapsing remitting **multiple sclerosis**.
- Patients receiving this antibody become lymphopenic and may also become neutropenic, anemic, and thrombocytopenic. As a result, patients should be closely monitored for opportunistic infections and hematologic toxicity.





DRUG	ACTION	ADVERSE EFFECTS
Alemtuzumab	Depletion of T lymphocytes	Cytokine-release syndrome; neutropenic, pancytopenia
Antithymocyte globulins	Destruction of T lymphocytes	Profound immunosuppression
Muromonab-CD3	Destruction of T lymphocytes	Cytokine-release syndrome
Cyclosporine	Blocks calcineurin and inhibits IL-2 synthesis	Nephrotoxicity, neurotoxicity, hepatotoxicity
Tacrolimus (FK506)	Blocks calcineurin and inhibits IL-2 synthesis	Nephrotoxicity, neurotoxicity, diabetes
Basiliximab	Blocks the IL-2 receptor	Gastrointestinal disorders
Daclizumab	Blocks the IL-2 receptor	Gastrointestinal disorders
Sirolimus	Blocks cytokine-stimulated cell proliferation	Hyperlipidemia, thrombocytopenia, leukopenia, headache, nausea
Everolimus	Blocks cytokine-stimulated cell proliferation	Hyperlipidemia, constipation, delayed wound healing, anemia
Azathioprine	Inhibits purine synthesis	Bone marrow suppression, hepatotoxicity, thrombocytopenia, anemia, neoplasia
Mycophenolate mofetil	Inhibits purine synthesis	GI upset, nausea, diarrhea, leukopenia, tumors, increased susceptibility to infection

Glucocorticoids

- The corticosteroids were the first pharmacologic agents to be used as immunosuppressives, both in transplantation and in various autoimmune disorders.
- They are still one of the mainstays for **attenuating rejection episodes**.
- The prolonged use of these agents is associated with **numerous adverse effects;** they are diabetogenic, can cause hypercholesterolemia, cataracts, osteoporosis and hypertension.

Consequently, efforts are being directed toward reducing or eliminating the use of steroids in the maintenance of allografts.

CASE STUDY

A 45-year-old man with high-risk acute myelogenous leukemia undergoes high-dose chemotherapy followed by an allogeneic stem cell transplant from an unrelated donor. He receives tacrolimus and low-dose methotrexate as prophylaxis for graft-vs-host disease. One month after blood count recovery, he develops a skin rash despite ongoing tacrolimus therapy. A skin biopsy confirms grade II acute graft-vs-host disease. How should this case be pharmacologically managed at this point?

CASE STUDY ANSWER

Glucocorticoids (steroids) are first-line treatment for acute graft-vs-host disease. Acute graft-vs-host disease is the process of donor T cells attacking host recipient tissues (including skin), despite ongoing immunosuppressive therapy such as tacrolimus. Adding a steroid (eg, prednisone) can ameliorate the T-cell response of graft-vs-host disease in most cases of grade II disease. See section in this chapter on Clinical Uses of Immunosuppressive Drugs.

The End