Proliferation Signal Inhibitors(PSIs)

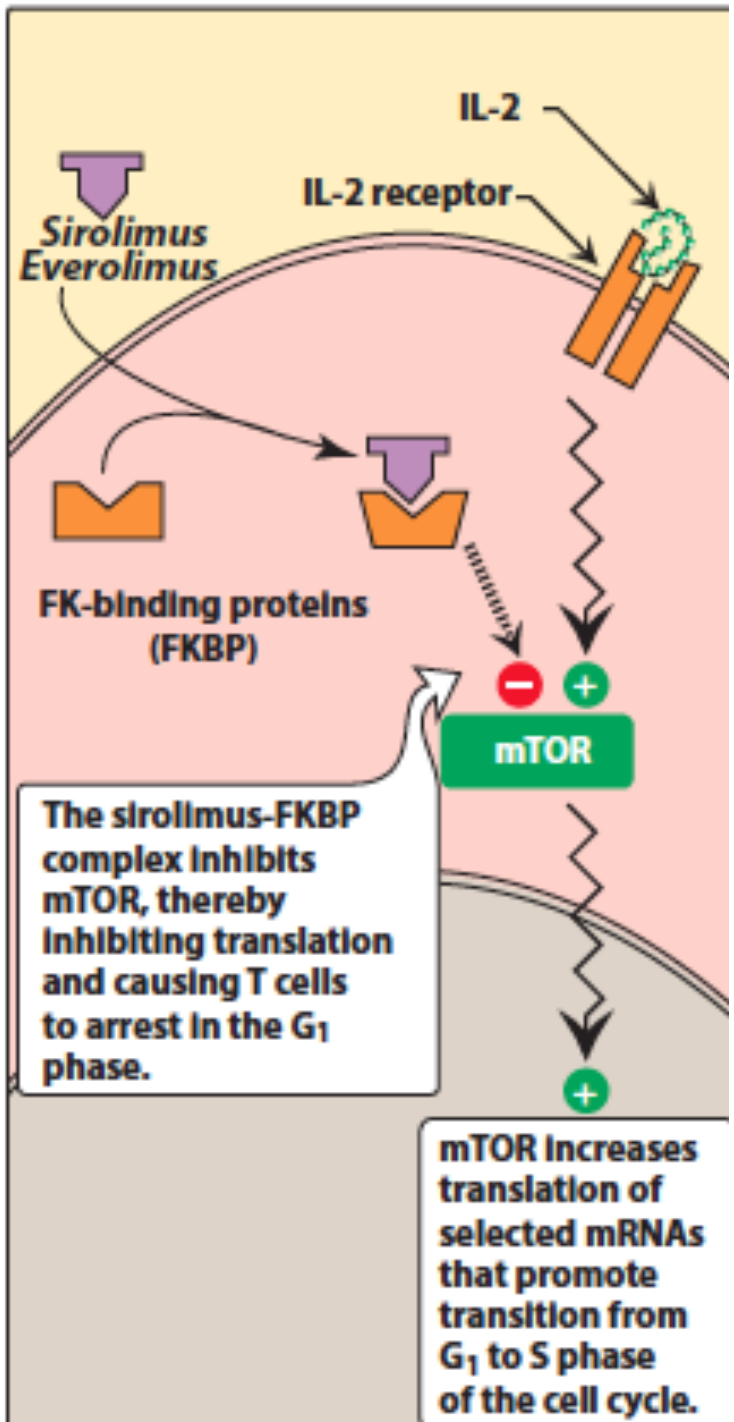
- Sirolimus(Rapamycin).
- Everolimus.
- Bind the circulating immunophylline FK506-binding protein12.
- Instead of forming a complex with calcineurin, sirolimus binds to mTOR (a serine/threonine kinase), interfering with signal 3.

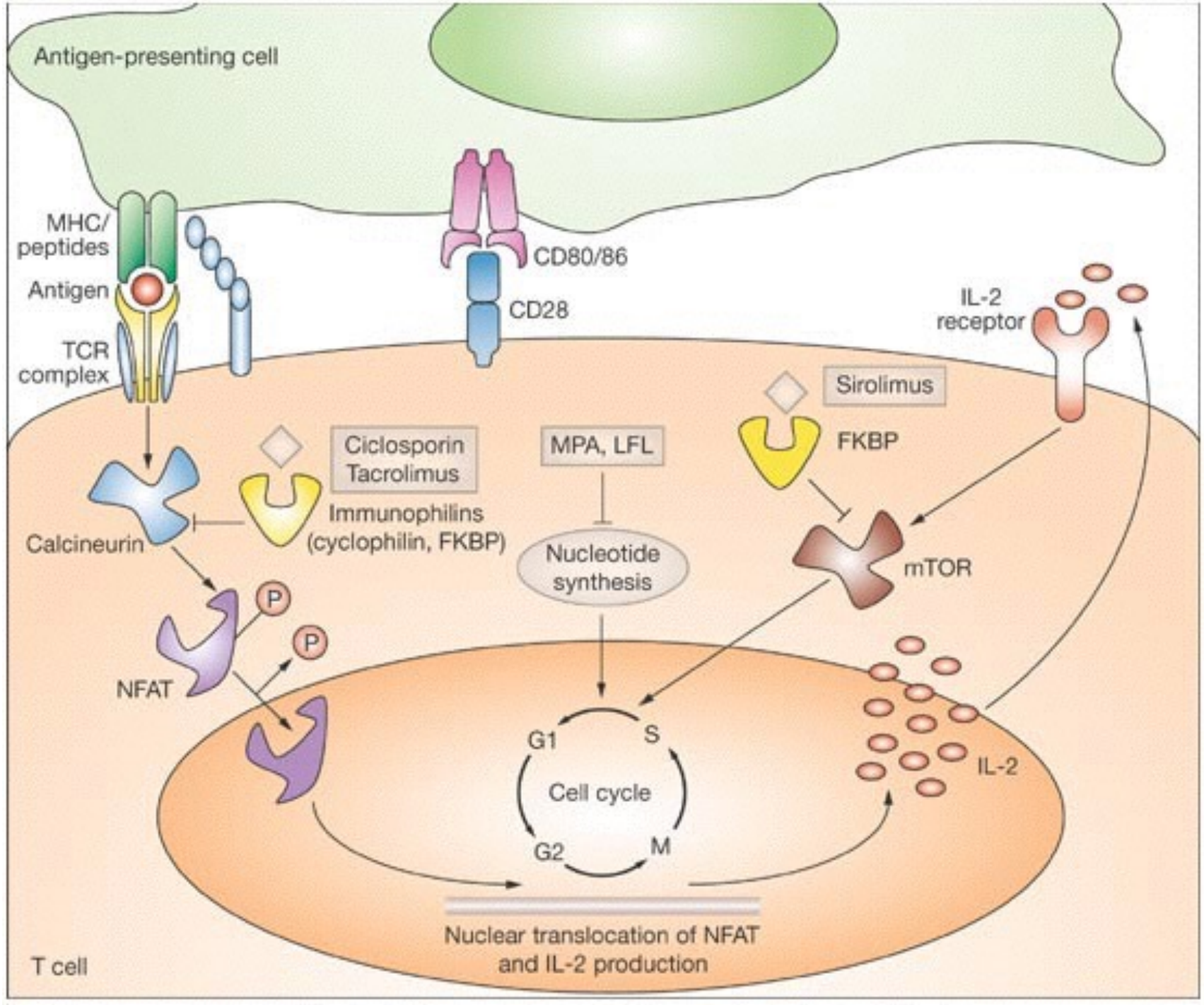
  –The complex inhibits interleukin-driven T-cell and B-cell proliferation as well as immunoglobulin production.

mTOR proteins

ToR proteins are essential for many cellular functions, such as cell cycle progression, DNA repair, and as regulators involved in protein translation.

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.


IL-2
IL-2 receptor
Sirolimus
Everolimus
FK-binding proteins
(FKBP)
mTOR
The sirolimus-FKBP complex inhibits mTOR, thereby inhibiting translation and causing T cells to arrest in the G_1 phase.
mTOR increases translation of selected mRNAs that promote transition from G_1 to S phase of the cell cycle.


Antigen-presenting cell
MHC/
peptides
Antigen
TCR
complex
CD80/86
CD28
IL-2
receptor
Sirolimus
FKBP
Ciclosporin
Tacrolimus
Immunophilins
(cyclophilin, FKBP)
MPA, LFL
Calcineurin
Nucleotide
synthesis
mTOR
P
P
NFAT
G1
S
Cell cycle
G2
M
IL-2
Nuclear translocation of NFAT
and IL-2 production
T cell

• Sirolimus(Rapamycin)& Everolimus.

- Available for oral and topical administration.
- Approved for use in renal transplantation, in combination with cyclosporine and corticosteroids
- **Sirolimus-eluting coronary stents:** The antiproliferative action of sirolimus is also valuable in cardiology where sirolimus-coated stents are used to inhibit restenosis of the blood vessels by reducing proliferation of the endothelial cells.
- Everolimus also indicated second-line treatment in patients with advanced renal cell carcinoma.

Adverse effects

- A common adverse effect of sirolimus is hyperlipidemia (elevated cholesterol and triglycerides), which may require treatment.
- The combination of cyclosporine and sirolimus is more nephrotoxic
- Others: headache, nausea and diarrhea, leukopenia, and thrombocytopenia.
- Impaired wound healing ……obese patients and those with diabetes.

Pharmacokinetics

- The drug is available as an oral solution or tablet.

- Although it is readily absorbed, high-fat meals can decrease the absorption.
- Sirolimus has a long half-life (57 to 62 hours), allowing for once-daily dosing.
- A loading dose is recommended at the time of initiation of therapy.
- Like both cyclosporine and tacrolimus, sirolimus is metabolized by the CYP3A4 isoenzyme, is a substrate for P-gp, and has similar drug interactions.
- Sirolimus also increases the concentrations of cyclosporine, and careful blood level monitoring of both agents must be done to avoid harmful drug toxicities.

Everolimus

- 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

- It has a much shorter half-life than sirolimus and requires twice-daily dosing.

- Everolimus increases drug concentrations of cyclosporine, thereby enhancing the nephrotoxic effects of cyclosporine, and is, therefore, recommended to be used with reduced doses of cyclosporine.

Everolimus side effects

- An additional adverse effect noted with everolimus is angioedema, which may increase with concomitant use of angiotensin-converting enzyme inhibitors.

- There is also an increased risk of kidney arterial and venous thrombosis, resulting in graft loss, usually in the first 30 days posttransplantation.

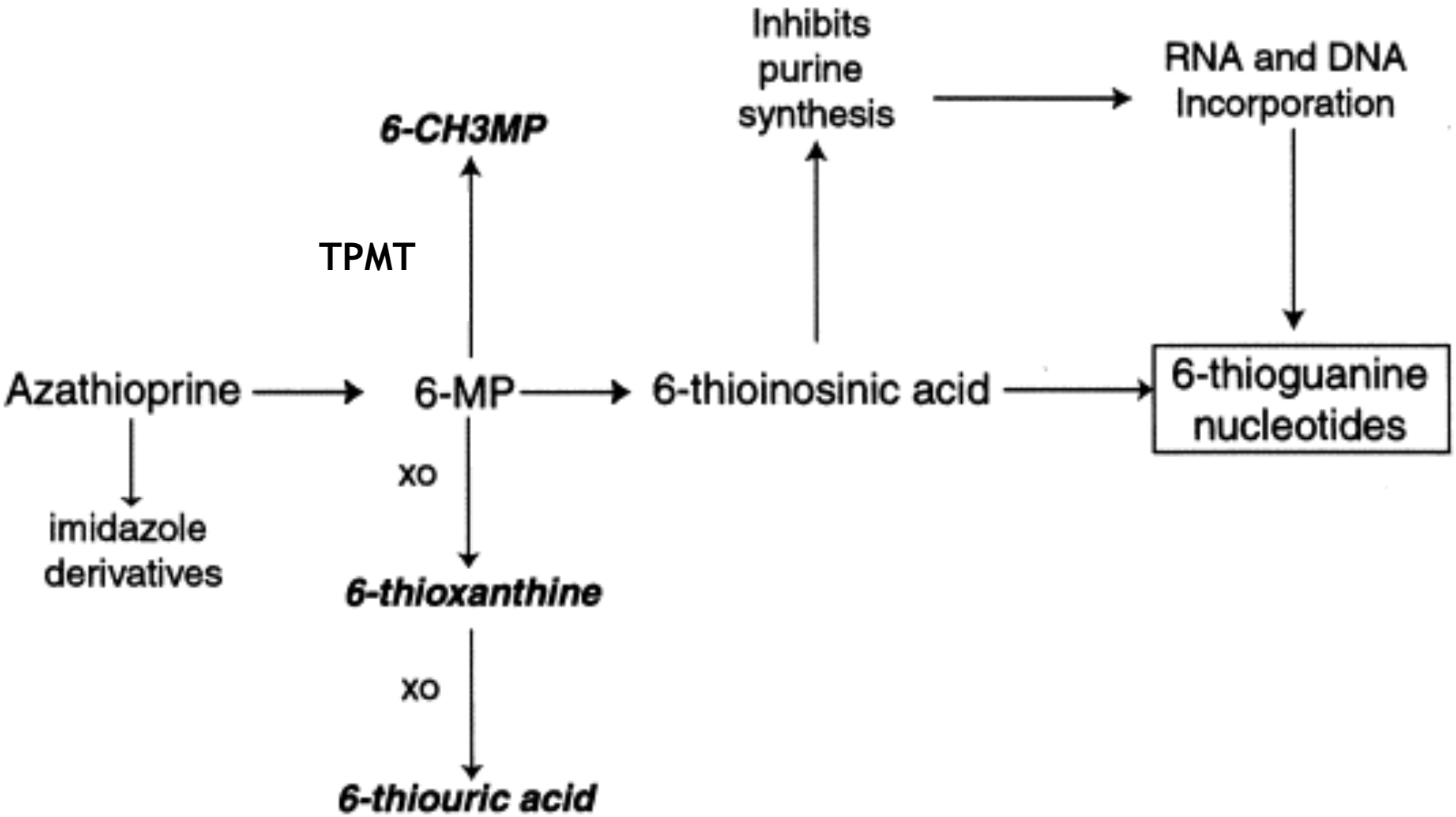
Cytotoxic Agents

- Azathioprine.
- Cyclophosphamide.
- Leflunomide.
- Hydroxychloroquine
- Others:
  - Vincristine.
  - Vinblastine
  - Methotrxate.
  - Cytarabine.
  - Pentostatin.

Azathioprine

- The first agent to achieve widespread use in organ transplantation.

- It is a prodrug that is converted first to 6-mercaptopurine (6-MP) and then to the corresponding nucleotide, thioinosinic acid.



TMPT: Thiopurine methyltransferase

Azathioprine

- The immunosuppressive effects of azathioprine are due to this nucleotide analog.

- Because of their rapid proliferation in the immune response and their dependence on the de novo synthesis of purines required for cell division, lymphocytes are predominantly affected by the cytotoxic effects of azathioprine.

- Its major nonimmune toxicity is **bone marrow suppression.**

Azathioprine

- Prodrug of mercaptopurine.
- Metabolized by Xanthine oxidase (so dose is reduced when given with allopurinol) .
- Antimetabolite: interferes with purine nucleic acid metabolism, and consequently will destroy and inhibit lymphoid cell proliferation stimulated by antigens.
- Blocks cellular immunity as well as primary and secondary serum antibody responses.

Azathioprine

- Used in renal allograft, acute glomerulonephritis, SLE, RA, Crohn's Disease, MS, and ITP.
- Toxicity:
  - Bone marrow suppression.
  - Skin rashes, fever.
  - N, V, D.
  - Hepatic dysfunction and jaundice.

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Drug Interactions

- Concomitant use with angiotensin-converting enzyme inhibitors or cotrimoxazole in renal transplant patients can lead to an exaggerated leukopenic response.

- Allopurinol, an agent used to treat gout, significantly inhibits the metabolism of azathioprine. Therefore, the dose of azathioprine must be reduced. Nausea and vomiting are also encountered.

Cyclophosphamide.

- Alkylating agent.
- Destroys proliferating lymphoid cells.
- Alkylates some resting cells.
- Large doses can induce an apparent specific tolerance to a new antigen if the drug is administered simultaneously with, or shortly after, the antigen.
- Toxicity: Pancytopenia, hemorrhagic cystitis, N, V, cardiac toxicity, electrolyte disturbances.

Mycophenolate mofetil

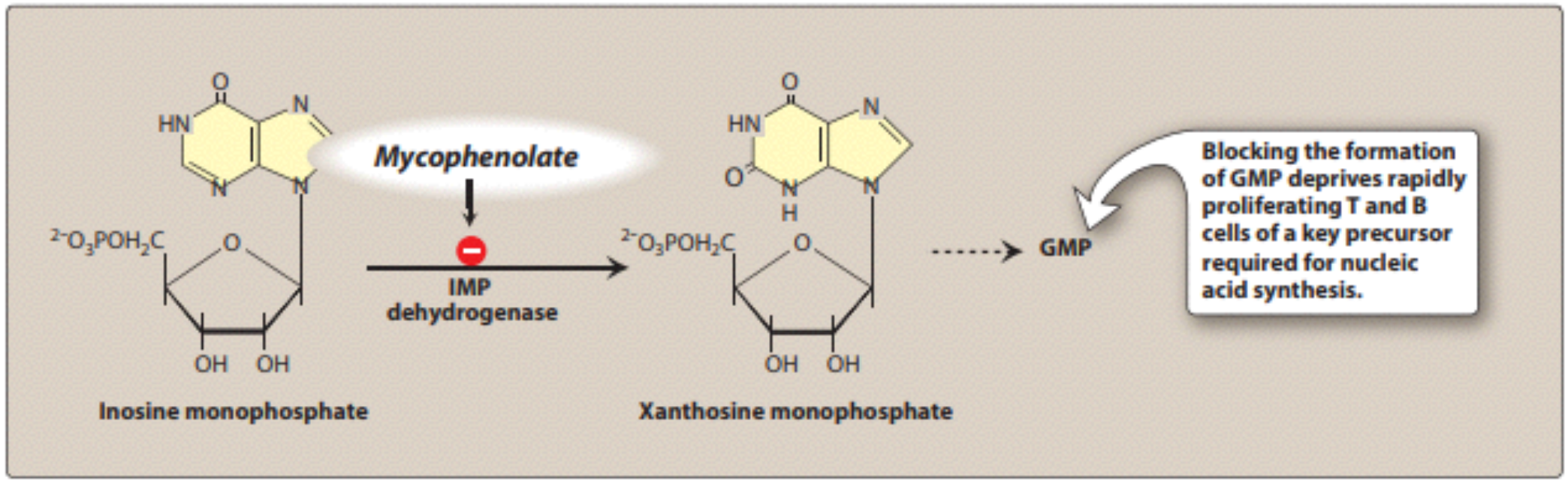
- Replaced azathioprine because of its safety and efficacy in prolonging graft survival.

- Uses: heart, kidney, and liver transplants.
- As an ester, it is rapidly hydrolyzed in the GI tract to mycophenolic acid.
- This is a potent, reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase, which blocks the de novo formation of guanosine phosphate.
- Deprives proliferating T and B cells of a key component of nucleic acids.

MYCOPHENOLATE

- More effective than Azathioprine in preventing acute rejection
- It is used in combination with cyclosporine and prednisolne
- Mycophenolate mofetil is used in solid organ transplant patients for refractory rejection and,

- In combination with prednisone, as an alternative to cyclosporine or tacrolimus in patients who do not tolerate those drugs.
- In renal transplants, it’s used with low-dose cyclosporine to reduced cyclosporine-induced nephrotoxicity.


Mycophenolate
IMP dehydrogenase
Inosine monophosphate
Xanthosine monophosphate
GMP
Blocking the formation of GMP deprives rapidly proliferating T and B cells of a key precursor required for nucleic acid synthesis.

Pharmacokinetics

- Mycophenolic acid is quickly and almost completely absorbed after oral administration.

- The glucuronide metabolite is excreted predominantly in urine.
- Concomitant administration with antacids containing magnesium or aluminum, or with cholestyramine, can decrease absorption of the drug.

Adverse effects

- GI adverse effects: (most Common) diarrhea, nausea, vomiting, and abdominal pain.

- High doses of mycophenolate mofetil are associated with a higher risk of CMV infection.

Thalidomide

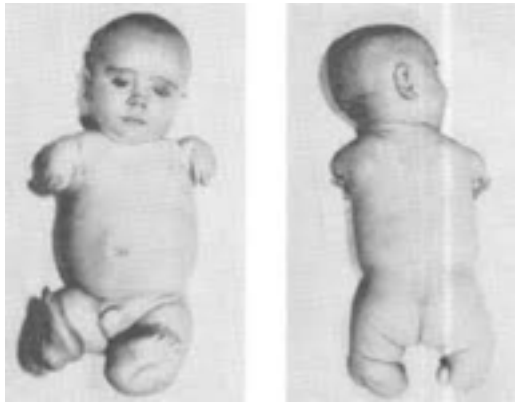
- Historical sedative drug withdrawn in 1960s because of its teratogenicity (Phocomelia).
- Inhibits angiogenesis.
- Antiinflammatory.
- Inhibits tumor necrosis factor-alpha(TNF-α)
- Reduces phagocytosis by neutrophils.
- Increases production of IL-10
- Enhances cell-mediated immunity via interaction with T cells.

Thalidomide

- Use continued only for leprosy.
- Very successful in multiple myeloma.
- Clinical trials in other diseases: myelodysplastic syndrome, AML, graft-versus-host disease, and solid tumors.

Thalidomide

- Toxicity:
  - Teratogenicity.
  - Peripheral neuropathy.
  - Constipation.
  - Rash.
  - Fatigue.
  - Hypothyroidism.
  - DVT.
- Lenalidomide
- CC-4047(Actimid).
  - Are much less toxic derivatives.



ANTIBODIES

- The use of antibodies has played a central role in prolonging allograft survival.

- They are prepared by immunization of either rabbits or horses with human lymphoid cells (producing a mixture of polyclonal antibodies or monoclonal antibodies).

Immunosuppresive Antibodies

- Hybridoma Technology, 1975.
- Molecular Biology >>>> Monoclonal Antibodies.
- Humanized Antibodies: “-umab” or “-umab”.
  - Replacing most of the regions, but keeping only the variable, antigen-specific regions intact.
- Chimeric Antibodies: “-imab” or “-ximab”.
  - Less complete replacement of the murine components.

Immunosuppresive Antibodies

- Antilymphocyte & Antithymocyte Antibodies.
- Muromonab.
- Immune Globulin Intravenous.
- Rh_o(D) Immune Globulin Micro-Dose.
- Hyperimmune Immunoglobulins.

Muromonab

Initial binding of *muromonab* to the antigen transiently activates the T cell and results in cytokine release (cytokine storm).

It is therefore customary to premedicate the patient with *methylprednisolone*, *diphenhydramine*, and *acetaminophen* to alleviate the cytokine release syndrome.

$Rh_o(D)$ Immune Globulin Micro-Dose.

- One of the earliest major advances in immunopharmacology.
- Concentrated (15%) solution of human IgG containing a higher titer of antibodies against the $Rh_o(D)$ antigen of the red cell.
- Given, to the mother, within 24-72 hours after the birth of an Rh-positive infant.
- Infant's red cells are cleared from circulation before the mother can generate a B-cell response against the $Rh_o(D)$ antigen.
- This will protect against future hemolysis.

Antitumor MABs

- Alemtuzumab.
- Bevacizumab.
- Ranibizumab.
- Cetuximab.
- Gemtuzomab.
- Panitumumab.
- Rituximab.
- Trastuzumab.
- Novilumab.

MABs to Deliver Isotopes to Tumors

- Arcitumomab.
- Capromab.
- Ibritumomab.
- Nofetumomab.
- Satomomab.
- Tositumomab

MABs used as Immunosuppressants and Antiinflammatory Agents

- Anti-TNF-Alpha MABs:
  - Adalimumab.
  - Etanercept
  - Infliximab
- Abatacept
- Alefacept
- Basiliximab
- Daclizumab
- Efalizumab
- Omalizumab
- Abiximab
- Eculizumab
- Palivizumab

Hybridoma

- Hybridoma technology (producing antigen-specific monoclonal antibodies). Hybridomas are produced by fusing mouse antibody-producing cells with tumor cells. Hybrid cells are selected and cloned, and the antibody specificity of the clones is determined.

- Clones of interest can be cultured in large quantities to produce clinically useful amounts of the desired antibody.

- Recombinant DNA technology can also be used to replace part of the mouse gene sequence with human genetic material, thus “humanizing” the antibodies and making them less antigenic.

- The names of monoclonal antibodies conventionally contain “xi” or “zu” if they are chimerized or humanized, respectively. The suffix “-mab” (monoclonal antibody) identifies the category of drug.

Anti-thymocyte globulins

- Polyclonal antibodies that are primarily used at the time of transplantation to prevent early allograft rejection along with other immunosuppressive agents.
- They may also be used to treat severe rejection episodes or corticosteroid-resistant acute rejection.
- The antibodies bind to the surface of circulating T lymphocytes, which then undergo various reactions, such as complement mediated destruction, antibody-dependent cytotoxicity, apoptosis, and opsonization.
- The antibody-bound cells are phagocytosed in the liver and spleen, resulting in lymphopenia and impaired T-cell responses.

Pharmacokinetics & Adverse effects

- Slowly infused intravenously
- Half-life extends from 3 to 9 days.

- Because the humoral antibody mechanism remains active, antibodies can be formed against these foreign proteins.

- Other adverse effects include chills and fever, leukopenia and thrombocytopenia, infections due to CMV or other viruses, and skin rashes.

Muromonab-CD3 (OKT3)

- Muromonab-CD3 is a murine (mouse) monoclonal antibody that is directed against the glycoprotein CD3 antigen of human T cells.

- Muromonab-CD3 was the first monoclonal antibody approved for clinical use in 1986, indicated for the treatment of corticosteroid-resistant acute rejection of kidney, heart, and liver allografts.
- 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.
- Basiliximab [is said to be “chimerized” because it consists of 25% murine and 75% human protein.
- “Humanized” monoclonal antibodies (for example, trastuzumab used for breast cancer, have a smaller stretch of nonhuman protein.

- Basiliximab is approved for prophylaxis of acute rejection in renal transplantation in combination with cyclosporine and corticosteroids. It is not used for the treatment of ongoing rejection.

Basiliximab

- An anti-CD25 antibody that binds to the α chain of the IL-2 receptor on activated T cells and, thus, interferes with the proliferation of these cells.
- Blockade of this receptor foils the ability of any antigenic stimulus to activate the T-cell response system.
- Basiliximab is given as an IV infusion. The serum half-life of basiliximab is about 7 days.
- 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.

MONOCLONAL ANTIBODIES (MABs)

Antitumor MABs

Alemtuzumab is a humanized IgG 1 with a kappa chain that binds to CD52 found on normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages, and a small population of granulocytes.

MONOCLONAL ANTIBODIES (MABs)

Currently, alemtuzumab is approved for the treatment of B-cell chronic lymphocytic leukemia in patients who have been treated with alkylating agents.

MABs Used to Deliver Isotopes to Tumors

Arcitumomab is a murine Fab fragment from an anti-carcinoembryonic antigen (CEA) antibody labeled with technetium 99m (99m Tc) that is used for imaging patients with metastatic colorectal carcinoma (immunoscintigraphy) to determine extent of disease.

CEA is often upregulated on tumor in patients with gastrointestinal carcinomas. The use of the Fab fragment decreases the immunogenicity of the agent so that it can be given more than once; intact murine monoclonal antibodies would elicit stronger HAMA.

MABs Used to Deliver Isotopes to Tumors

Capromab pendetide is a murine monoclonal antibody specific for prostate specific membrane antigen. It is coupled to isotopic indium (111 In) and is used in immunoscintigraphy for patients with biopsy-confirmed prostate cancer and post-prostatectomy in patients with rising prostate specific antibody level to determine extent of disease.

MABs Used to Deliver Isotopes to Tumors

Ibritumomab tiuxetan is an anti-CD20 murine monoclonal antibody labeled with isotopic yttrium (90 Y).

The radiation of the isotope coupled to the antibody provides the major antitumor activity.

Ibritumomab is approved for use in patients with relapsed or refractory low-grade, follicular, or B-cell non-Hodgkin's lymphoma, including patients with rituximab-refractory follicular disease. It is used in conjunction with rituximab in a two-step therapeutic regimen.

Cytokines

- Are a large and heterogeneous group of proteins with diverse functions.
- Mediate their effects through receptors, like hormones.
- May have antiproliferative, antimicrobial, and antitumor effects.
- Produced using gene cloning techniques.
- Have very short half lives, and given sc.
- All induce fever, flu-like symptoms, anorexia, fatigue, and malaise.

Cytokines

- Interferones: INF- α, β, and γ.
- Interleukins: 1-35.
- Tumor Necrosis factor: α, and β.
- Granulocyte colony-stimulating factor.
- Granulocyte-macrophage colony-stimulating factor.
- Macrophage colony-stimulating factor.
- Erythropoietin.
- Thrombopoietin

Cytokines

- **Interferon (INF): INF-α,β,γ**
  - Antiviral, anticancer, immunomodulating effects.
  - Antiviral effects : INF-α,β > INF-γ
  - immunomodulating effects: INF-γ
  - Adverse Effects: flu-like symptoms, fatigue, malaise
- **Interleukin-2 (IL-2)**
  - T cell proliferation, T_H, NK, LAK cell activation
  - Treatment of malignant melanoma, renal cell carcinoma, Hodgkin disease
  - Adverse Effects: fever, anorexia, etc .

Cytokine Inhibitors

- **Anakinra:**
  - Is a recombinant form of the naturally occurring IL-1 receptor antagonist.
  - Approved for adult rheumatoid arthritis