26

Immunology

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Alia

Proliferation Signal Inhibitors (PSIs)

•Sirolimus (Rapamycin)

•Everolimus.

•Bind the circulating immunophilin FK506-binding protein12.

• Instead of forming a complex with calcineurin, sirolimus binds to mTOR (a serine/threonine kinase), interfering with signal 3.

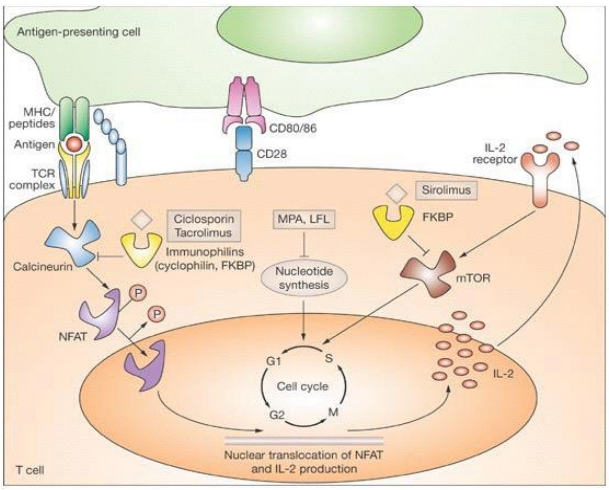
The complex inhibits interleukin-driven T-cell and B-cell proliferation as well as immunoglobulin production.

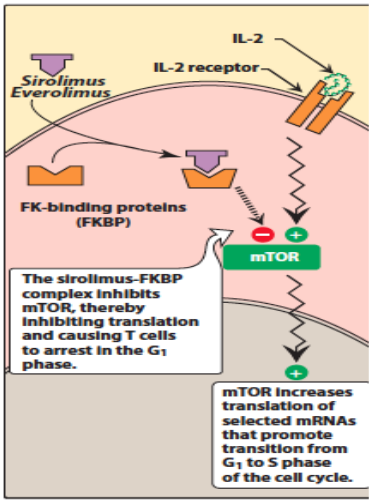
mTOR protein (target of Rapamycin)

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

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

Remember that mTOR is activated by signal 3.





Sirolimus and everolimus bind to immunophilins (FKBP12), and this complex binds to mTOR which is a serine-threonine kinase and blocks the signal 3 (instead of binding to calcineurin as cyclosporine or tacrolimus).

By comparing the action of Sirolimus, Everolimus (mTOR inhibitors) with cyclosporine, tacrolimus: They have the same intracellular binding protein (FKBP), but the target after the drug-protein complex is formed is different.

Sirolimus (Rapamycin)& Everolimus.

Available for oral and topical administration.

Approved for use in renal transplantation, in combination with cyclosporine and corticosteroids.

SPECIAL use of sirolimus is STENTS.

So, we have stents that release of sirolimus.

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.

EXTRA: stents are tubular placed temporarily inside blood vessels, canal, or duct to aid healing or relieve obstruction.

Everolimus also indicated second-line treatment in patients with **advanced renal cell carcinoma.**

ADVERSE EFFECT:

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. MONITOR KIDNEY FUNCTION.

Others: **headache, nausea and diarrhea, leucopenia** (all of immunosuppressant agents cause leucopenia) and **thrombocytopenia** (because these immunosuppressant agents block the proliferation of the cells).

Impaired wound healing (also because It suppress proliferation of the cells) specifically obese patients and those with diabetes.

Note: the treatment of elevated level of cholesterol (LDL) is statin (Lipitor).

MOA: statin inhibits HMG-COA reductase (inhibits the rate limiting step of cholesterol synthesis), So, by decreasing the amount of endogenous cholesterol in the liver, it will uptake cholesterol from the blood.

Statin also has other effects, **Statins** inhibit **Rho kinase pathway,** leading to dilation of blood vessels.

REMEMBER: *Rho/Rho* kinase signal transduction *pathway* is one of the principal mechanisms of *vasoconstriction.*

This dilation can be beneficial in vascular disease such as coronary heart disease.

Statin is metabolized by cytochrome p 450. But we have also sirolimus which is a substrate of cytochrome p450, so this will lead to drug- drug interaction. **we said that sirolimus causes hyperlipidemia in the patients thus requires treatment.** So, if we give them statin, this will lead to competition between these drugs at the same enzyme (decreasing the metabolism of them) result in increasing the level of each one. IT IS NECESSARY TO MONITOR THE LEVEL OF THEM.

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 (57-62 hours) allowing for once daily dosing.

A loading dose is recommended at the time of initiation of therapy. (we often use loading dose (doubling of the dose to reach above minimal effective concentration to give the effect earlier). (BY DEFAULT, MONITOR THE TOXICITY).

Like both cyclosporine and tacrolimus, sirolimus is metabolized by the cyp3A4 isoenzyme and is a substrate for P-gp (cause less absorption which leads to different bioavailability between individuals because the difference of expression of genes) 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 effect:

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 post transplantation.

Cytotoxic Agents:

Azathioprine, Cyclophosphamide, Leflunomide, Hydroxychloroquine, Vincristine, Vinblastine, Methotrexate, Cytarabine, Pentostatin.

Azathioprine is discovered by George Herbert Hitching **and Gertrude Elion in 1957**

Azathioprine: The first agent to achieve widespread use in organ transplantation (anti-inflammatory).

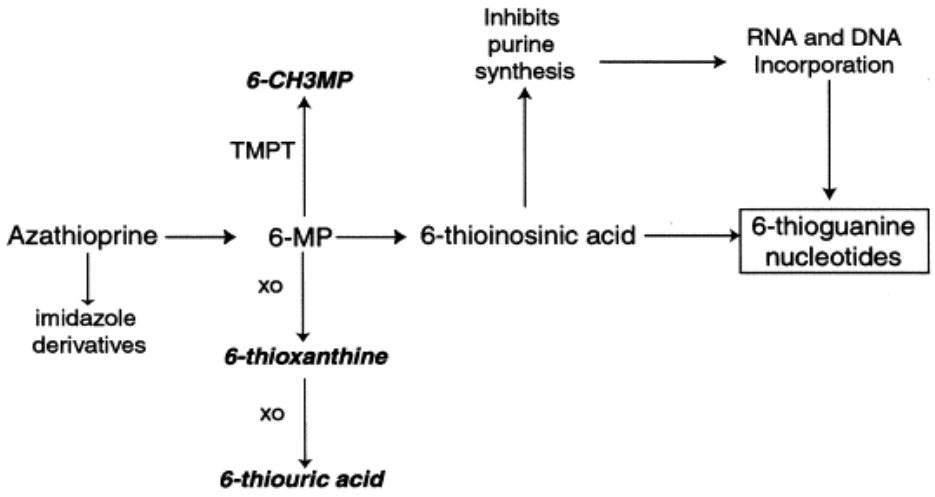
It is a prodrug that is converted first to 6- mercaptopurine (6-MP) and then to the corresponding nucleotide, thioinosinic acid.

MOA: 6-MP is converted into thioinosinic acid which inhibits the purine synthesis by its converging 6-thioguanine nucleotide so once it is incorporated to DNA or RNA result in leading to stop the growing chain.

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 (there is no salvage pathway in T lymphocyte), lymphocytes are predominantly affected by the cytotoxic effects of azathioprine.

Its major nonimmune toxicity is bone marrow suppression.



TMPT: Thiopurine methyltransferase

Metabolized by Xanthine oxidase (so, the dose of azathioprine must be reduced when given with allopurinol). *allopurinol is an inhibitor of xanthine oxidase".

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 response.

Azathioprine is used in inflammatory bowel disease because we use a type that is not absorbed, allowing it to stay for long time in small intestine to do its function.

Used in renal allograft, acute glomerulonephritis, SLE, RA, Crohn's Disease, MS, and ITP.

Toxicity:

Bone marrow suppression, Skin rashes, Fever, Hepatic dysfunction and Jaundice, N, V, D (nausea, vomiting, diahrrea).

Drug interaction:

Concomitant use with angiotensin-converting enzyme inhibitors (ACE) or cotrimoxazole(antibiotic) in renal transplant patients can lead to an exaggerated leukopenia response.

Allopurinol, an agent used to treat gout, significantly inhibits the metabolism of azathioprine or 6-MP by inhibition of xanthine oxidase , so level of 6-MP will increase and the toxicity increases.

Therefore, the dose of azathioprine must be reduced. Nausea and vomiting are also encountered.

Cyclophosphamide: cytotoxic agent.

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 (deficiency of all three cellular components of blood(RBC, WBC, platelets)) , hemorrhagic cystitis N, V, cardiac toxicity, electrolyte disturbances.

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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.

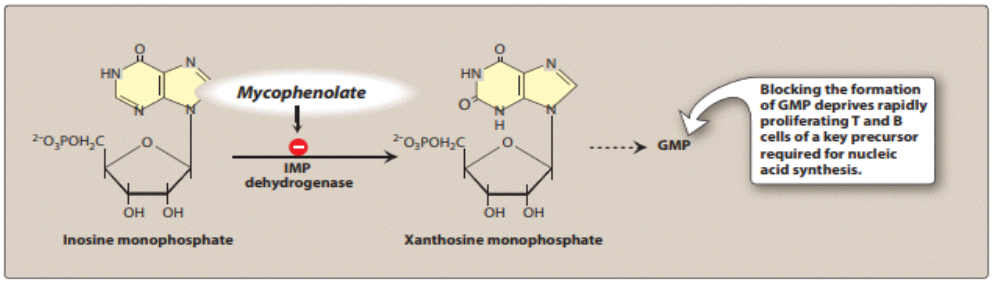
Deprives proliferating T and B cells of a key component of nucleic acids.

More effective than Azathioprine in preventing acute rejection.

It is used in combination with cyclosporine and prednisolone(glucocorticoid).

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 reduce cyclosporine-induced nephrotoxicity.



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 (lipid lowering drug), can decrease absorption of the drug.

Adverse effect:

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

High doses of mycophenolate mofetil are associated higher risk of the opportunistic infection and **CMV infection**.

Thalidomide:

Historical sedative drug withdrawn in 1960s because of its teratogenicity (Phocomelia).

Anti-inflammatory.

Inhibits tumor necrosis factor-alpha (TNF-α).

Enhances cell-mediated immunity via interaction with T cells. (sedative, antiemetic effect).

Use continued only for leprosy, very successful in multiple myeloma.

Clinical trials in other diseases: myelodysplastic syndrome, AML, graft-versushost.

Toxicity:

Teratogenicity, Peripheral neuropathy, Constipation, Rash, Fatigue,

Hyperthyroidism, DVT (Deep Vein Thrombosis).

Thalidomide: was given to the pregnant women in 1960s, but these women gave birth to child without limbs. And this drug was discontinued until the end of the last century …Some studies were done and noticed that it has immunosuppressant property.

So it is used with caution (when a female need to use it (has certain kind of tumor or multiple myeloma), she should use at least 2 type of contraception).

Other form of drug:

Lenalidomide, CC-4047(Actimid)

Are much fewer toxic derivatives **, but they are still teratogenic.**

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ANTIBODY: the use of antibodies has played an important role against large group of disease, ex: **In cancer**: they either kill the target cancer cells or deliver the radioactive material to the cancer tissue.

In musculoskeletal system, some antibodies have been used in autoimmune disease such as (rheumatoid arthritis).

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).

EXTRA: recall the differences between the polyclonal and monoclonal antibodies:

*Polyclonal antibodies are a heterogeneous mix of antibodies, derived from the immune response of multiple B-cells, and each one recognizes a different epitope on the same antigen.

Because polyclonal antibodies are composed of a mixture of antibodies that represents the natural immune response to an antigen, they are prone to a higher risk of batch-to-batch variability than monoclonal antibodies.

*Monoclonal antibodies come from a single B-cell parent clone and therefore only recognize a single epitope per antigen. These B-cells are immortalized by fusion with hybridoma cells, allowing for long-term generation of identical monoclonal antibodies.

Because monoclonal antibodies specifically detect a particular epitope on the antigen, they are less likely than polyclonal antibodies to cross-react with other proteins.

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Immunosuppressive Antibodies:

Hybridoma Technology,1975.

Molecular Biology >>>> Monoclonal antibody.

Humanized Antibodies: "-umab". Replacing most of the regions, but keeping only the variable, antigenic- specific regions intact.

Chimeric Antibodies: "- less complete replacement of the murine-component. imab" or "-ximab"

murine: related to mouse.

Hybridoma: is an immunosuppressant agent used to inhibit certain receptor to suppress the immunity.

But there is a problem: NO complete compatibility between the antibodies of human and those from animals (mouse) which leads to IMMUNOGENICITY.

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.

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

IMMUNOSUPPRESIVE ANTIBODY:

Antilymphocyte & Antithymocyte Antibodies.

Muromonab.

Immune Globulin Intravenous.

Rho(D) Immune Globulin Micro-Dose.

Hyperimmune Immunoglobulins .

Muromonab-CD3 (OKT3).

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.

For more clarification:

Muromonab-CD3 is a **murine (mouse)** monoclonal antibody that is directed against the glycoprotein CD3 antigen of human thymocytes and mature T cell to kill human cytotoxic T cells. Because it is murine (comes from mouse) antibody, **the initial use of this drug** lead to an antigenic reaction that cause activation of T cells and release an excessive number of cytokines (cytokines storm).

Because of that effect, we give the patient immunosuppressant agents such as (with methylprednisolone, diphenhydramine(antihistamine), glucocorticoid and acetaminophen (to decrease the inflammatory process). But this drug has been discontinued because its antigenicity, also we have developed the humanized antibody.

Muromonab-CD3 was the first monoclonal antibody approved for clinical indicated for the treatment corticosteroid-resistant acute rejection of kidney, heart, and liver allograft.

The drug has been discontinued from the market due to the availability of newer biologic drugs with similar efficacy and fewer side effects.

Rho(D) Immune Globulin Micro-Dose: One of the earliest major advances in immunopharmacology.

Concentrated (IV 15%) solution of **human IgG** containing a higher titer of antibodies against the Rho(D) antigen of the red cell.

Given, to the mother, within 24-72 hours after the birth of a Rh-positive infant.

Infant's red cells are cleared from circulation before the mother can generate a B-cell response against the Rho.

This will protect against future hemolysis.

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Anti-thymocyte globulins:

Polyclonal antibodies that are primarily used at the time of transplantation to prevent early allograft rejection along with another immunosuppressive agent.

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 and Adverse Effects.

Slowly infused intravenously.

Half-life extends from 3-9 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.

immunoglobulins IV: they are **polyclonal pool human Ig** against many diseases **(**autoimmune disorder, HIV, bone marrow transplantation, SLE, ITP).

THEY DO NOT HAVE SPECIFIC ANTIGENS, so we can use them in different conditions.

Hyper Ig: (pool of **selected human and animal** donors with higher titer of antibodies against **particular agents** such as venom, viruses, toxin, rat snake venom, Korean venom, CMP, RCP, varicella zoster, herpes.

we give that as a vaccine. So, it is a combination of antibody which is given to people who travel to places where the disease is common, or to increase the immunity.

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

•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.

Antitumor MABs

Alemtuzumab is a **humanized IgG1** with a kappa chain that bind to CD52 found on normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages, and a small population of granulocytes.

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 ant carcinoembryonic antigen (CEA) antibody labeled with **technetium** 99m (99 m Tc) that is used for **imaging patients with metastatic colorectal carcinoma (immunoscintigraphic) 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 (human anti-mouse antibody).

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 immunoscintigraphic for patients with biopsy-confirmed prostate cancer and post-prostatectomy in patients with rising prostate specific antibody level to determine extent of disease.

Ibritumomab dioxetane is an anti-CD20 murine monoclonal antibody labeled with isotopic yttrium(Y90).

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.

NOTE: The Doctor didn't have enough time to cover all slides.

Good Luck