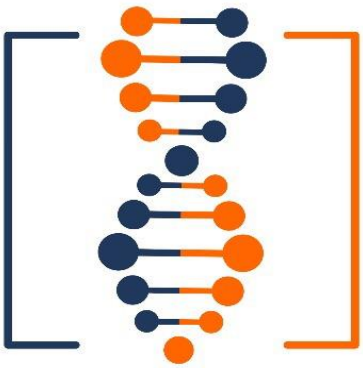
DESTINATION
PHARMAGENS

Cell mediated immune response

- The CD4 molecule associated with T cell receptor on helper T cells anchors the major histocompatibility complex class II (MHC II) carrying the antigen peptide so that it is able to activate the T cell receptor
- Stimulation of T cell receptor phosphorylates **Phospholipase c**,
- This **Phospholipase c** hydrolyses **PIP2** to generate **DAG** and **IP3**.
- While DAG activates PKc to produce MAPkinase dependent and other actions, **IP3** releases intracellular **Ca2**+.
- After binding to calmodulin this Ca2+ activates a membrane associated serine/ threonine phosphatase called **calcineurin**
- This **calcineurin** dephosphorylates regulatory protein **'nuclear factor of activated T-cell' (NFAT),** permitting its intranuclear migration and transcription of cytokine genes leading to **production of IL-2** along with other **interleukins**, **GM-CSF, TNFα , interferon** etc.
- **IL-2 is the major cytokine for T-cell multiplication and differentiation.**
- The **mTOR** is an important link in the cascade of signalling pathways which lead to proliferation and differentiation of T-cells activated by IL-2 and other cytokines.
- TNFα is secreted by activated macrophages and other immune cells to act on TNF receptors (TNFR1, TNFR2) which are located on the surface of neutrophils, fibroblasts, endothelial cells as well as found in free soluble form in serum and serous fluids. TNFα amplifies immune inflammation by releasing other cytokines and enzymes like collagenases and metalloproteinases.
- Stimulated macrophages and other mononuclear cells releases **IL-1** which activates helper T-cells and induces production of other ILs, metalloproteinases.

CALCINEURIN INHIBITORS (Specific T-cell inhibitors)

Cyclosporine

It is a cyclic polypeptide with 11 amino acids

Physiological effects

- It profoundly and selectively inhibits T lymphocyte proliferation, IL-2 and other cytokine production as well as response of inducer T cells to IL-1, without any effect on suppressor T-cells.
- Lymphocytes are arrested in G0 or G1 phase.
- Cyclosporine is most active when administered before antigen exposure, but can, in addition, suppress the responses of primed helper T cells; hence useful in autoimmune diseases as well.
- Cyclosporine selectively suppresses cell mediated immunity (CMI), prevents graft rejection and yet leaves the recipient with enough immune activity to combat bacterial infection.
- Unlike cytotoxic immunosuppressants, it is free of toxic effects on bone marrow and RE system.
- Humoral immunity remains intact.

MOA

- Cyclosporine enters target cells and binds to **cyclophilin**, an immunophilin class of protein.
- The complex then binds to calcineurin and inactivates calcineurin → response of the helper T cell to antigenic stimulation fails.
- Cyclosporine also enhances expression of **transforming growth factor β (TGF β),** an inhibitor of IL-2 which attenuates IL-2 stimulated T-cell proliferation and production of killer lymphocytes.

ADVERSE EFFECTS

- However, it is nephrotoxic—the major limitation, and impairs liver function.
- Other effects are sustained rise in BP, precipitation of diabetes, anorexia, lethargy, hyperkalaemia, hyperuricaemia, opportunistic infections, hirsutism, gum hyperplasia, tremor and seizures.

USES

- Cyclosporine is the most effective drug for prevention and treatment of **graft rejection reaction**.
- It is routinely used in renal, hepatic, cardiac, bone marrow and other **transplantations**.
- When graft rejection has started, it can be given i.v, **Blood level monitoring is required for effective therapy.**
- **Cyclosporine is a second line drug in autoimmune diseases**
- **It is generally used along with corticosteroids or Mtx.**

DRUG INTERACTIONS

- All nephrotoxic drugs like aminoglycosides, vancomycin, amphotericin B and NSAIDs enhance its toxicity. By depressing renal function, it can reduce excretion of many drugs.
- Potassium supplements and K+ sparing diuretics can produce marked hyperkalaemia in patients on cyclosporine.

Tacrolimus (FK506)

- It binds to a different cytoplasmic immunophilin protein labelled '**FK 506 binding protein (FKBP)**', but the subsequent steps are the same, i.e. inhibition of helper T cells via calcineurin.
- Tacrolimus is administered orally as well as by i.v. infusion. Therapeutic application, clinical efficacy as well as toxicity profile are similar to cyclosporine.
- **Tacrolimus also requires blood level monitoring for dose adjustment. However, due to higher potency and easier monitoring of blood levels, it is generally preferred now for organ transplantations.**
- Tacrolimus may be useful in patients whose rejection reaction is not suppressed by cyclosporine. It is particularly valuable in liver transplantation because its absorption is not dependent on bile. Being more potent, it is also suitable for suppressing acute rejection that has set in.

- Hypertension, hirsutism, gum hyperplasia and hyperuricaemia are less marked than with cyclosporine, but Dose limiting toxicity is renal.

mTOR INHIBITORS

Sirolimus

- This new and potent immunosuppressant is a macrolide antibiotic
- It binds to the same immunophillin **FKBP** as tacrolimus, but the sirolimus-FKBP complex inhibits another kinase called 'mammalian target of rapamycin' (mTOR), and does not interact with calcineurin.
- The mTOR is an important link in the cascade of signalling pathways which lead to proliferation and differentiation of T-cells activated by IL-2 and other cytokines.
- Sirolimus arrests the immune response at a later stage than cyclosporine.

USES

For prophylaxis and therapy of graft rejection reaction, sirolimus can be used alone, but is generally combined with lower dose of cyclosporine/tacrolimus and/or corticosteroids and mycophenolate mofetil. The latter combination avoids use of a calcineurin inhibitor, and is particularly suitable for patients developing renal toxicity with cyclosporine.

ADVERSE EFFECTS

sirolimus can suppress bone marrow, mainly causing thrombocytopenia. Rise in serum lipids is common. Other adverse effects are diarrhoea, liver damage and pneumonitis.

Everolimus

It is similar to sirolimus in mechanism, clinical efficacy, doses, toxicity and drug interactions

ANTIPROLIFERATIVE DRUGS

(Cytotoxic immunosuppressants)

Certain cytotoxic drugs used in cancer chemotherapy exhibit prominent immunosuppressant property, mainly by preventing clonal expansion of T and B lymphocytes

Azathioprine

- Possess marked immunosuppressant action, may be due to its selective uptake into immune cells and intracellular conversion to the active metabolite 6-mercaptopurine, which then undergoes further transformations to inhibit de novo purine synthesis and damage to DNA of immune cells.

- It selectively affects differentiation and function of **T cells** and inhibits **cytolytic lymphocytes**; **CMI** is primarily depressed.
- The most important application of azathioprine is prevention of renal and other graft rejection, but it is less effective than cyclosporine; generally combined with it or used in patients developing cyclosporine toxicity.
- it is frequently employed for maintening remission in inflammatory bowel disease
- It may be an alternative to long-term steroids in some other autoimmune diseases as well.

Methotrexate

- This folate antagonist is a potent immunosuppressant which markedly depresses cytokine production and cellular immunity, and has antiinflammatory property.
- It has been used as a first line drug in many autoimmune diseases like rapidly progressing rheumatoid arthritis, severe psoriasis, pemphigus, myasthenia gravis, uveitis, chronic active hepatitis.

Cyclophosphamide

- This cytotoxic drug has more marked effect on B cells and humoral immunity compared to that on T cells and CMI.
- It has been particularly utilized in bone marrow transplantation in which a short course with high dose is generally given.
- In other organ transplants it is employed only as a reserve drug.

Chlorambucil

It has relatively weak immunosuppressant action which is sometimes utilized in autoimmune diseases and transplant maintenance regimens.

Mycophenolate mofetil (MMF)

- It is a newer immunosuppressant; prodrug of mycophenolic acid which selectively inhibits **inosine monophosphate dehydrogenase**, an enzyme essential for de novo synthesis of guanosine nucleotides in the T and B cells (**these cells, unlike others, do not have the purine salvage pathway**). Lymphocyte proliferation, antibody production and CMI are inhibited.
- As **'add on'** drug to cyclosporine + glucocorticoid in renal transplantation, it has been found as **good or even superior to azathioprine, but should not be combined with azathioprine.**
- MMF + glucocorticoid + sirolimus is a non-nephrotoxic combination that is utilized in patients developing renal toxicity with other immunosuppressant combination.
- Vomiting, diarrhoea, leucopenia and predisposition to CMV infection, g.i. bleeds are the prominent adverse effects.

Glucocorticoids

- Glucocorticoids have potent immunosuppressant and anti-inflammatory action
- They particularly inhibit MHC expression and activation/proliferation of T lymphocytes. Expression of several IL and other cytokine genes is regulated by corticosteroids and production of adhesion molecules is depressed.
- The shortlived rapid lymphopenic effect of steroids is due to sequestration of lymphocytes in tissues.
- Accordingly, they have marked effect on CMI but little effect on humoral immunity.
- The corticosteroids are widely employed as companion drug to cyclosporine or other immunosuppressants in various organ transplants.
- In case graft rejection sets in—large doses of corticoids i.v. are employed for short periods.
- They are used in practically all cases of severe autoimmune diseases, especially during exacerbation.
- Long term complications are the greatest limitations of steroid use; and it is maintenance of remission for which other immunosuppressants often prove safer.

BIOLOGICAL AGENTS

These are biotechnologically produced recombinant proteins or polyclonal/monoclonal antibodies directed to cytokines or lymphocyte surface antigens which play a key role in immune response.

TNF α inhibitors

TNFα is **secreted by activated macrophages and other immune cells** to act on TNF receptors (TNFR1, TNFR2) which are located on the surface of neutrophils, fibroblasts, endothelial cells as well as found in free soluble form in serum and serous fluids. TNFα **amplifies immune inflammation** by **releasing other cytokines** and enzymes like collagenases and metalloproteinases.

Etanercept

- This fusion protein of human TNF receptor and Fc portion of human IgG1 neutralizes both TNF α and TNF β.
- It prevents activation of macrophages and T-cells during immune reaction.
- It is used mostly in combination with Mtx in rheumatoid arthritis patients who fail to respond adequately to the latter.

Infliximab

- It is chimeral monoclonal antibody against TNFα which binds and inactivates TNα
- it has proven useful in refractory rheumatoid arthritis, fistulating Crohn's disease, ulcerative colitis, psoriasis and ankylosing spondylitis.

Adalimumab

- It is fully human recombinant anti-TNF α antibody indicated in the same range of autoimmune diseases as infliximab, and like the latter, does not bind TNF β, but is less antigenic.
- It can be added to Mtx or other conventional drugs for additional benefit.

IL-1 receptor antagonist

Stimulated macrophages and other mononuclear cells elaborate IL-1 which activates helper T-cells and induces production of other ILs, metalloproteinases.

Anakinra

- This recombinant human IL-1 receptor antagonist prevents IL-1 binding to its receptor and has been approved for use in refractory rheumatoid arthritis not controlled by conventional DMARDs.
- Anakinra along with continued Mtx has been used alone as well as added to TNF antagonists, because its clinical efficacy as monotherapy appears to be lower.

IL-2 receptor antagonist

Daclizumab

- It is a highly humanized chimeric monoclonal anti CD-25 antibody which binds to and acts as IL-2 receptor antagonist.
- Combined with glucocorticoids, calcineurin antagonists and/or azathioprine/MMF, it is used to prevent renal and other transplant rejection reaction.

Basiliximab

- This is another anti CD-25 antibody with higher affinity for the IL-2 receptor
- Clinical use of basiliximab is similar to that of daclizumab.
- **Both daclizumab and basiliximab can cause anaphylactic reactions and promote opportunistic infection.**

Anti-CD3 antibody

Muromonab CD3

- It is a **murine monoclonal antibody** against the CD3 glycoprotein expressed near to the T cell receptor on helper T cells
- Binding of muromonab CD3 to the CD3 antigen obstructs approach of the MHCII antigen complex to the T-cell receptor. Consequently, antigen recognition is interfered, and participation of T-cells in the immune response is prevented.

- Following antibody binding, the T-cell receptor is internalized and the T-cells get rapidly depleted from blood, partly by cytolysis and partly by their migration to non-lymphoid organs. An immune blocked state results.
- Muromonab CD3 is now primarily used for acute transplant rejection reaction, particularly in steroid-resistant cases.
- Initial doses of muromonab CD3 are associated with 'cytokine release syndrome' with flu-like symptoms, viz. chills, rigor, high fever, wheezing, malaise, etc. which is due to release of TNF, ILs and interferon.
- The symptoms decrease in severity with subsequent doses.
- Occasionally aseptic meningitis, intragraft thrombosis, life-threatening pulmonary edema, seizures and a shock-like state are produced.

Polyclonal antibodies

Anti-thymocyte globulin (ATG)

- It is a polyclonal antibody purified from horse or rabbit immunized with human thymic lymphocytes which contains antibodies against many CD antigens as well as HLA antigens.
- It binds to T lymphocytes and depletes them. It is a potent immunosuppressant and has been used primarily to suppress acute allograft rejection episodes,
- Used in steroidresistant cases, by combining with other immunosuppressants, including steroids.

Anti-D immune globulin

- It is human IgG having a high titer of antibodies against Rh (D) antigen. It binds the Rho antigens and does not allow them to induce antibody formation in Rh negative individuals.
- It is used for prevention of postpartum/post-abortion formation of antibodies in Rho-D negative, DU negative women who have delivered or aborted an Rho-D positive, DU positive baby/foetus.
- Administered within 72 hours of delivery/ abortion, such treatment prevents Rh haemolytic disease in future offspring.
- Higher doses (1000–2000 µg) are needed for Rh negative recipients of inadvertantly administered Rh positive blood. It should never be given to the infant or to Rho-D positive, DU positive individuals.

IMMUNOSUPPRESSION IN ORGAN TRANSPLANTATION

Use of immunosuppressants is essential for successful organ transplantation. In general 3 types of regimens are used depending upon the stage of transplantation.

1. Induction regimen

This is given in the perioperative period: starting just before the transplant to about 2–12 weeks after it. Accelerated rejection develops in the first week, while acute rejections are most likely from 2–12 weeks.

The most common regimens include **triple therapy** with cyclosporine/tacrolimus/ sirolimus + prednisolone + MMF/azathioprine.

The sirolimus + prednisolone + MMF combination avoids risk of renal toxicity.

Two drug and single drug regimens are also used.

2. Maintenance regimen

This is given for prolonged periods, may be life-long.

Triple drug regimen consisting of maintenance doses of any three of the following choices—cyclosporine/ tacrolimus, sirolimus, prednisolone, azathioprine/ MMF

Nephrotoxicity is often the limiting factor with cyclosporine/tacrolimus, while long-term steroid therapy has its own problems.

3. Antirejection regimen

This is given to suppress an episode of acute rejection.

Steroid pulse therapy (methylprednisolone 0.5–1 g i.v. daily for 3–5 days) is effective in majority of cases.

In case of no response, muromonab CD3/ ATG is given as rescue therapy or the antibodies are combined with steroids.

Tacrolimus, sirolimus, MMF have also been used in rescue therapy of steroid resistant rejection.

Adverse effects

(a) Increased risk of bacterial, fungal, viral (especially CMV) as well as opportunistic infections.

(b) Development of lymphomas and related malignancies after a long latency.

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