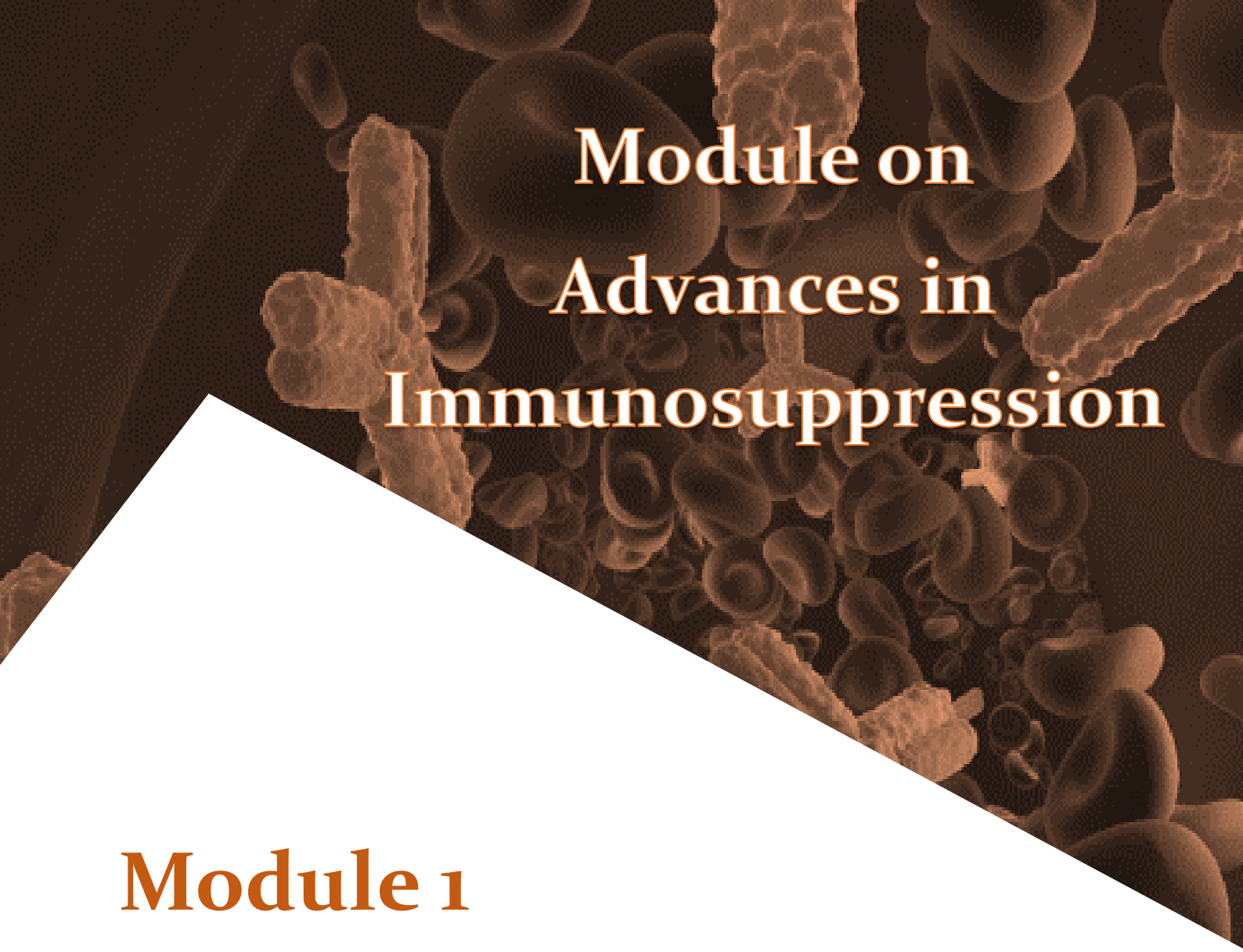

Module on Advances in Immunosuppression

Module 1

Understanding Pharmacology of Immunosuppressive Drugs

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Introduction

Immunosuppressive agents have a long history, with a recent surge in their development. In 1949, Nobel Prize laureate Philip Hench discovered that the corticosteroid cortisone had significant anti-inflammatory effects in patients with rheumatoid arthritis (RA). In the early 1960s, independent discoveries by Calne, Murray, and Zukoski revealed that azathioprine (AZA) was effective in preventing kidney allograft rejection. Despite these breakthroughs, many mechanisms of the immune response remained unclear.

During the 1960s and 1970s, cyclophosphamide, originally developed for cancer chemotherapy, was repurposed for immune diseases and transplantation. Antilymphocyte serum also gained popularity as a lymphocyte-depleting agent in kidney transplantation. The late 1970s and early 1980s marked a revolutionary period with the development of monoclonal antibodies (mAbs) for human therapeutic use and the discovery of the immunosuppressive effects of cyclosporin A from fungal fermentation extracts of Tolypocladium inflatum.

The 1990s saw significant advancements in immunosuppressive drug development. Increased understanding of B and T cell development, activation, and proliferation, cytokine and chemokine signaling, and complement activation led to the creation of targeted therapeutics, particularly humanized mAbs. Drug discovery during this period also enhanced the understanding of immune response mechanisms. Similar to cyclosporin A, sirolimus (previously called rapamycin) was initially developed as an antifungal. It was later found to possess antineoplastic and immunosuppressive properties, which were eventually linked to the mammalian target of rapamycin (mTOR) pathways.

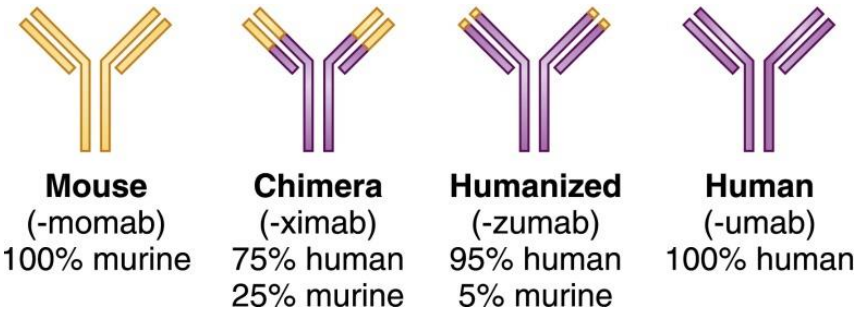

Figure 1. Schematic representation and nomenclature of mAbs in clinical use

In recent decades, the rapid pace of immunosuppressive drug development from the late 1990s has slowed, but progress continues steadily. Enhancements in the efficacy and specificity of existing agents have made it increasingly challenging to develop new drugs that meet the high standards for superiority and safety required for regulatory approval and public acceptance.

This challenge is particularly evident in nephrology, where immunosuppressive agents are often used for rare, orphan diseases that are difficult to study in large multicenter trials. Consequently, many new agents that nephrologists encounter will be used off-label, based on experiences from other fields such as rheumatology and oncology. However, there are notable exceptions, with emerging efforts to address inflammation in acute kidney injury (AKI) and maintenance hemodialysis.

The main classes of immunosuppressive drugs used in clinical practice to prevent tissue rejection include calcineurin inhibitors, TOR inhibitors, cytotoxic agents, glucocorticoids, and monoclonal antibodies. These drugs typically need to be used for life and come with significant side effects.

Cyclosporine

In the 1970s, the genesis of specific immunosuppressive therapy occurred with the discovery of cyclosporine by J. F. Borel in 1976. Initially explored as an antifungal agent, its immunoregulatory properties were swiftly recognized. This breakthrough emerged from a research initiative of a Swiss pharmaceutical company aimed at uncovering novel antibiotics from fungal metabolites. Cyclosporine was derived from the fungus Tolypocladium inflatum Gams, initially isolated from a soil sample in Norway. Chemical analysis revealed cyclosporine to be a cyclic undecapeptide. Its structure and conformation were elucidated through methods including chemical degradation, X-ray crystallography, and nuclear magnetic resonance imaging. Cyclosporine is characterized as a hydrophobic compound, insoluble in water yet soluble in various organic solvents.

Mechanism of Action

Cyclosporine penetrates the cell membrane and binds to cyclophilins found in the cytoplasm. Cyclophilins constitute a family of small proteins with a high affinity for cyclosporine and its active analogs. While they are predominantly abundant in lymphoid cells, they are also present in various human tissues. The interaction between cyclosporine and cyclophilin forms a complex known as cyclosporine–cyclophilin complex.

During an immune response, activation of the T-cell receptor triggers an elevation in intracellular Ca2+ levels, leading to the activation of calcineurin, a calcium-dependent serine/threonine phosphatase enzyme. Under normal physiological conditions, calcineurin, upon activation by Ca2+, dephosphorylates a cytosolic component of the nuclear factor of activated T cells (NFATc). Subsequently, dephosphorylated NFATc translocates from the cytoplasm to the nucleus, where it associates with the nuclear component of NFAT (NFATn). This association of NFATc and NFATn initiates the transcriptional activation of numerous genes, including those encoding cytokines such as IL-2, IL-3, IL-4, TNFα, GM-CSF, and others.

Following the administration of cyclosporine, a complex forms between cyclosporine and cyclophilin, which subsequently binds to calcineurin. This interaction inhibits calcineurin's ability to dephosphorylate NFATc, preventing its translocation to the nucleus and subsequent association with NFATn. This association between NFATc and NFATn is crucial for initiating

the production of IL-2, achieved through their binding to the promoter of the interleukin-2 (IL-2) gene. Consequently, the production of IL-2, essential for optimal immune response function, is inhibited. It's noteworthy that cyclosporine doesn't hinder cytokine-induced transduction mechanisms and has no impact on T cell antigen recognition in conjunction with MHC molecules.

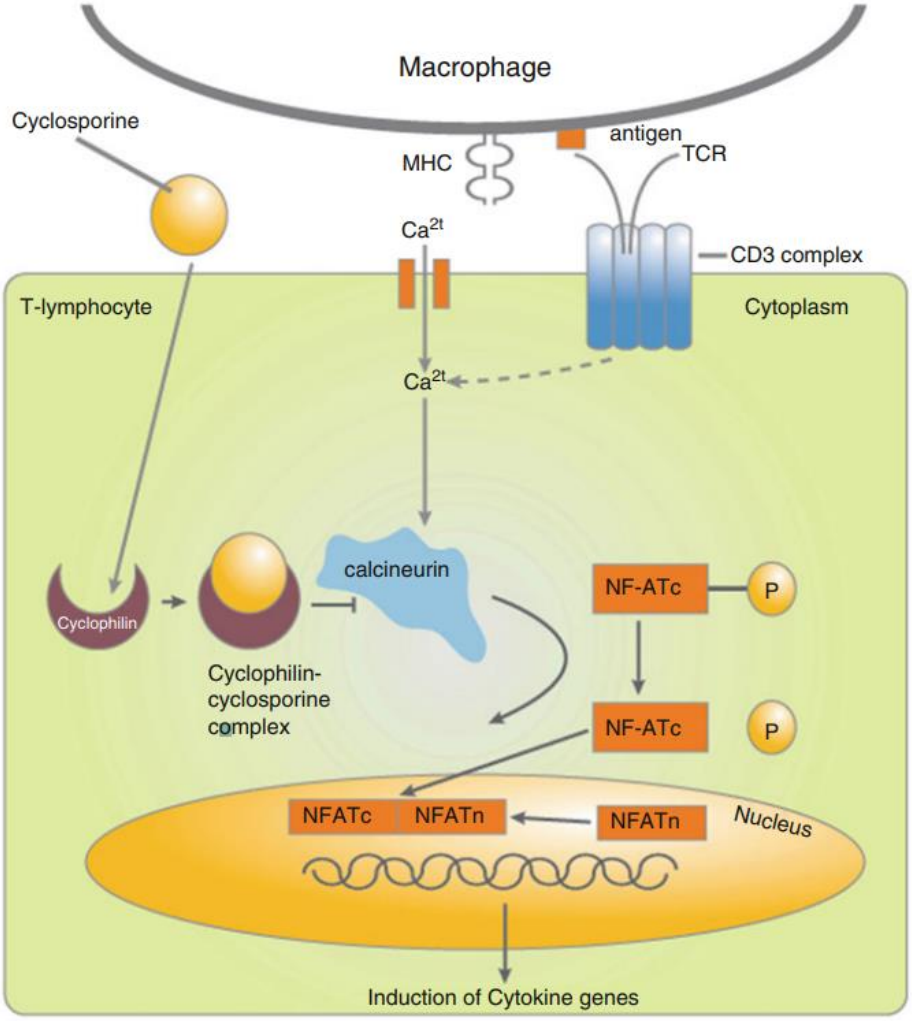

Figure 2. Mechanism of action of cyclosporine: Cyclosporine easily penetrates the cytoplasm of target cells, where it binds to cyclophilins. This binding forms a stable complex between cyclosporine and cyclophilin, which then binds to calcineurin, inhibiting its activity. Calcineurin is a calcium-dependent enzyme, a serine/threonine phosphatase that, upon activation by calcium ions (Ca2+), dephosphorylates a cytosolic component of NFAT (NFATc, cytosolic factor of activated T cells). Following dephosphorylation, NFAT migrates from the cytoplasm to the nucleus, where it associates with NFATn (nuclear factor of activated T cells) and triggers the transcription of several cytokine genes, including IL-2. Cyclosporine inhibits

calcineurin activity upon binding to cyclophilins, consequently inhibiting IL-2 production and the expression of other cytokines.

Absorption, Distribution, and Excretion

Cyclosporine is administered orally or intravenously, with its oral bioavailability ranging from 20% to 50%. Peak plasma concentrations are typically reached within 3 to 4 hours post-administration. Approximately 70% of the drug binds to erythrocytes, while leukocytes contain about 15–20% of cyclosporine despite their low presence in total blood volume. Notably, cyclosporine is extensively distributed in compartments beyond the vasculature, potentially contributing to some of its toxic side effects. The half-life of cyclosporine is approximately 6 hours. Primarily metabolized in the liver by CYP3A, it is excreted in bile, with only minimal amounts appearing in urine. Although over 20 metabolites of cyclosporine have been identified, they exhibit significantly lower pharmacologic activity and toxicity compared to the parent drug. Inhaled cyclosporine has been utilized post-lung transplantation to mitigate undesired side effects, potentially delaying the onset of obliterative bronchiolitis.

Drug Interactions

Blood concentrations of cyclosporine can be influenced by medications that affect microsomal enzymes, especially the CYP3A system. Drugs that inhibit this enzyme can decrease the metabolism of cyclosporine, leading to elevated blood concentrations. Examples of such drugs include antifungal agents, antibiotics, glucocorticoids, calcium channel blockers, protease inhibitors, among others. Conversely, drugs that enhance CYP3A activity can accelerate the metabolism of cyclosporine, resulting in decreased blood concentrations. Examples of these drugs include phenytoin, phenobarbital, trimethoprim–sulfamethoxazole, and rifampin.

Toxicity

The primary adverse effects associated with cyclosporine include renal impairment and nephrotoxicity, which manifest in approximately 25–75% of patients. These effects can lead to reduced glomerular filtration rate and renal plasma flow, along with damage to proximal tubules and endothelial cells of small blood vessels. Cyclosporine also induces hyperuricemia, potentially exacerbating hypercholesterolemia, increasing P-glycoprotein activity, and worsening gout. Hypertension is prevalent among most cardiac transplant recipients and around 50% of renal transplant recipients. Approximately half of patients receiving

cyclosporine experience elevated hepatic transaminase levels or plasma bilirubin concentrations. Hirsutism and gingival hypoplasia are observed in 10–30% of patients on cyclosporine therapy. Other adverse effects may include peptic ulcers, pancreatitis, gum hyperplasia, convulsions, respiratory difficulties, fever, vomiting, and confusion. While there is an increased susceptibility to fungal and viral infections, the incidence of malignancies is low when cyclosporine is administered alone without other immunosuppressive agents.

Clinical Uses

Cyclosporine is employed to forestall organ rejection following tissue transplantation and is further utilized in the treatment of conditions like rheumatoid arthritis, psoriasis, and dry eyes (keratoconjunctivitis sicca). In tissue transplantation scenarios, it is predominantly administered alongside other immunosuppressive agents, with dosage adjustments contingent upon individual clinical requirements. However, its potential for nephrotoxicity necessitates careful consideration, particularly prior to tissue grafting. This presents a particular challenge in renal transplant cases, where distinguishing between rejection and renal toxicity is imperative.

Tacrolimus

In 1984, tacrolimus, a 23-membered lactone chain, was isolated from Streptomyces tsukubaensis, despite its initial discovery in a soil fungus.

Mechanism of Action

Tacrolimus exerts its effects by suppressing peptidyl-prolyl isomerase activity through binding with the immunophilin FK506-binding protein-12 (FKBP-12). This complex formed between tacrolimus and FKBP-12 subsequently binds to calcineurin, leading to the inhibition of calcineurin phosphatase activity. Consequently, calcineurin loses its ability to dephosphorylate NFATc, preventing its translocation to the nucleus, where its association with NFATn is crucial for the activation of vital cytokine genes. Hence, tacrolimus operates similarly to cyclosporine, albeit by binding to a distinct set of immunophilins within the cytoplasm. Like cyclosporine, tacrolimus effectively curtails the secretion of key cytokines and inhibits T-cell activation.

Absorption, Distribution, and Excretion

Tacrolimus is administered orally, typically in a twice-daily dose regimen, or via injection. To address issues of noncompliance, particularly prevalent in solid transplant recipients at risk of acute graft rejection, a modified release (MR) oral formulation of tacrolimus has been developed, enabling once-daily dosing. Absorption of tacrolimus in the gastrointestinal (GI) tract is incomplete and variable. It exhibits high plasma protein binding, ranging from 75% to 99%, and has a half-life of approximately 12 hours. Predominantly metabolized in the liver by the enzyme CYP3A, tacrolimus generates metabolites with immunosuppressive activity. The majority of tacrolimus is eliminated through fecal excretion, with less than 1% excreted in urine without undergoing significant metabolism.

Drug Interactions

Similar to cyclosporine, drugs affecting the CYP3A system can influence the blood concentration of tacrolimus. Inhibitors of this enzyme can elevate tacrolimus levels in the blood, while drugs that enhance CYP3A activity may reduce its concentration. When combined with cyclosporine, tacrolimus may induce additive renal toxicity.

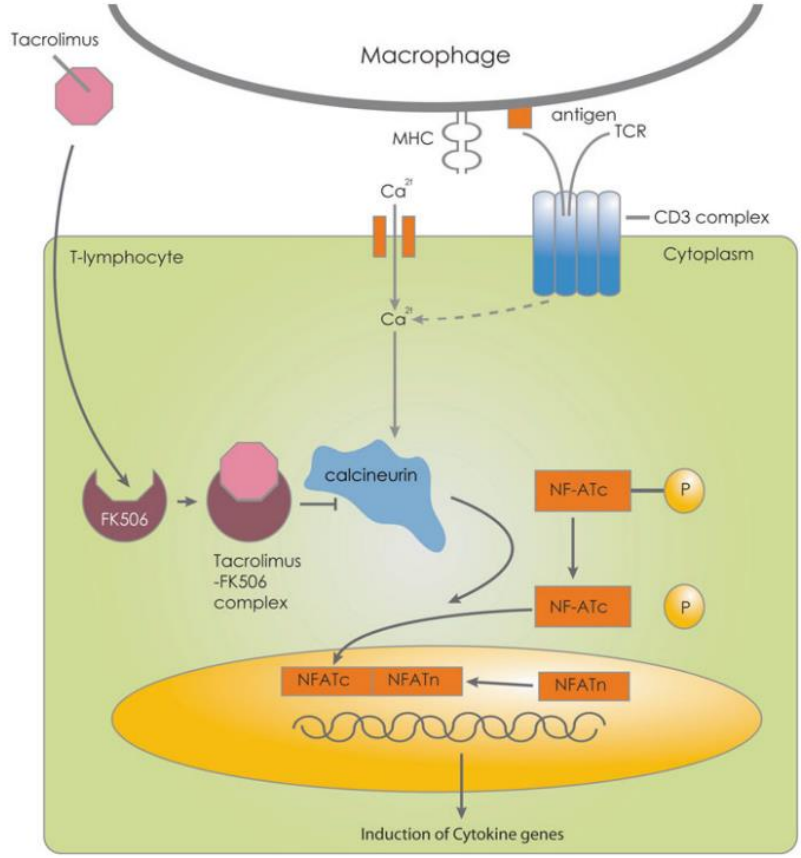

Figure 3. The mechanism of action of tacrolimus: Tacrolimus readily penetrates the cytoplasm of target cells, where it binds to immunophilins (FK506-BP). This complex formed between tacrolimus and immunophilins stably associates with calcineurin, effectively inhibiting its activity. Calcineurin, a calcium-dependent enzyme and serine/threonine phosphatase, is activated by calcium ions (Ca2+) and dephosphorylates a cytosolic component of NFAT (NFATc, cytosolic factor of activated T cells). Upon dephosphorylation, NFATc translocates from the cytoplasm to the nucleus, where it associates with NFATn (nuclear factor of activated T cells) and initiates the transcription of various cytokine genes, including IL-2. Tacrolimus impedes calcineurin activity following its association with immunophilins, thereby inhibiting IL-2 production and the synthesis of other cytokines.

Toxicity

Side effects linked to tacrolimus administration encompass nephrotoxicity, hepatotoxicity, hypertension, tremors, seizures, diabetes mellitus, neuropathy, blurred vision, depression, loss of appetite, and confusion. Tacrolimus usage may also predispose individuals to opportunistic infections and exacerbate pre-existing infections, including fungal or viral (herpes) infections. Notably, it does not impact LDL cholesterol or uric acid levels. Similar to cyclosporine, combining tacrolimus with other immunosuppressive agents may heighten the risk of tumor development.

Clinical Uses

Tacrolimus shares immunosuppressive characteristics with cyclosporine, but its potency surpasses that of cyclosporine, necessitating lower doses. Its primary application involves preventing rejection following allogeneic transplants to mitigate the risk of organ rejection. Additionally, tacrolimus is prescribed for patients exhibiting signs of rejection despite cyclosporine treatment. It is also employed in the management of severe atopic dermatitis and refractory uveitis.

Sirolimus (Rapamycin)

Sirolimus, a macrolide antibiotic (macrocyclic lactone), was initially discovered in soil samples from Easter Island, originating from the bacterium Streptomyces hygroscopicus. It bears structural similarities to tacrolimus.

Mechanism of Action

Sirolimus binds to the cytosolic protein FK-binding protein R (FKBP-12) but does not inhibit calcineurin activity. Unlike cyclosporine and tacrolimus, sirolimus does not bind to cyclophilins, the cytosolic receptors for cyclosporine. Instead, after binding to its cytosolic receptors, sirolimus inhibits a protein kinase, the mammalian target of rapamycin (mTOR) pathway, by suppressing protein phosphatase 2 (PP2-A). This inhibition of mTOR prevents cells from progressing to the S phase, resulting in cell cycle arrest. Consequently, sirolimus blocks T-cell proliferation, but its effects occur downstream of the interleukin-2 receptors.

Sirolimus induces cytotoxicity through the generation of reactive oxygen species (ROS), which regulate Bak protein expression and cause mitochondrial dysfunction. G2/M phase cell cycle arrest results from decreased expressions of CDK2 and cyclin B1. The rapamycin-receptor complex binds to mammalian target of rapamycin (mTOR) or FK506-binding protein-12-rapamycin-associated protein 1 (FRAP1), encoded by the mTOR gene. Mammalian target of rapamycin (mTOR) is a serine threonine kinase and is a member of phosphatidylinositol-3-kinase (PI3K)-related kinases (PIKKS). mTOR is a downstream effector of the PI3K/AKT pathway and serves as the catalytic subunit of mTORC1 and mTORC2, two structurally distinct complexes present in different subcellular compartments.

Rapamycin inhibits mTORC1 to exert its effects, but its effects on mTORC2 are more complex, as the mTORC2 signaling pathway is not yet well-defined. Rapamycin inhibits T-cell proliferation in response to various cytokines, including IL-1, IL-2, IL-3, IL-4, IL-6, IGF, PDGF, and colony-stimulating factor.

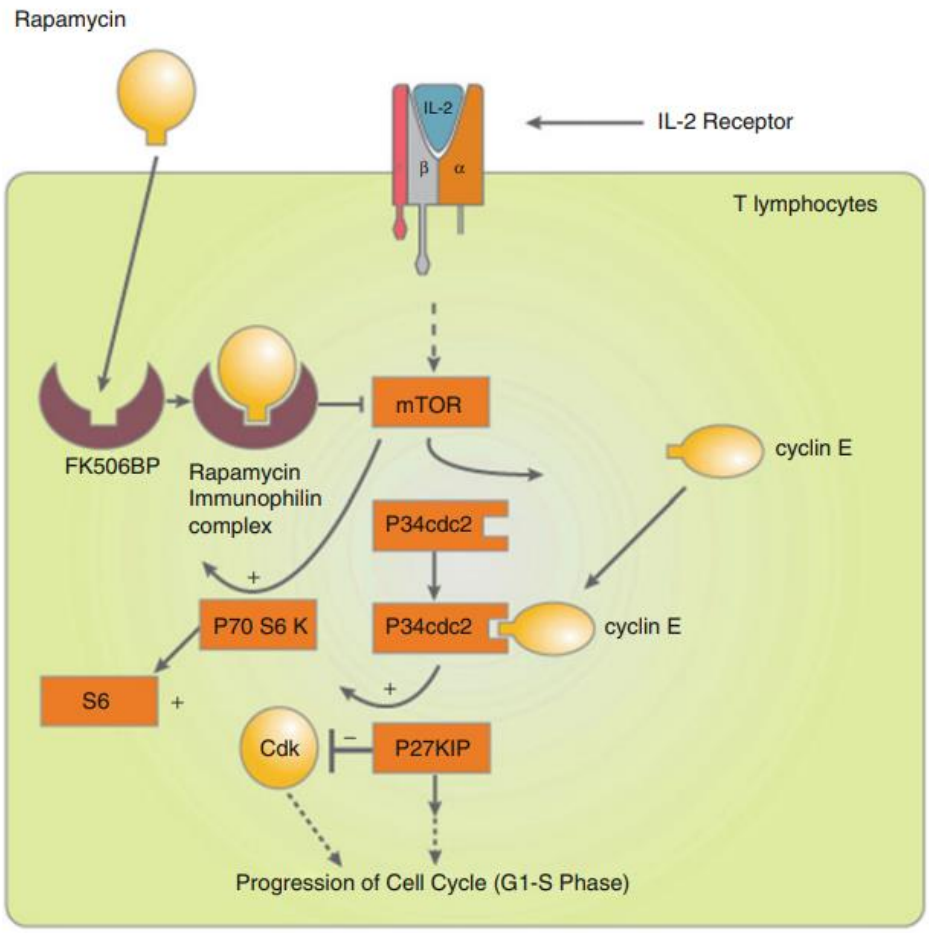

Figure 4. The mechanism of action of sirolimus: Sirolimus efficiently enters the cytoplasm of target cells, where it binds to immunophilins (FK506-BP). However, unlike other immunosuppressive agents, the sirolimus-immunophilin complex does not inhibit calcineurin activity. Instead, it binds to the mammalian target of rapamycin (mTOR). This complex halts cell cycle progression from the G1 to S phase. Sirolimus targets various components including the eukaryotic initiation factor (eIF-4 F), 70 KD S6 protein kinase (p70S6k), and multiple cyclin-dependent kinases (cdk). Consequently, it disrupts downstream signaling pathways initiated after IL-2 receptor activation, thereby inhibiting T-cell proliferation.

Absorption, Distribution, and Excretion

Sirolimus is swiftly absorbed following oral administration, with peak blood levels attained approximately one hour later in healthy individuals. However, kidney transplant patients experience a doubled time to reach peak blood concentrations. Its systemic availability is approximately 15%, and the presence of high-fat meals can hinder its bioavailability. Around 40% of sirolimus is bound to plasma proteins. For transplant patients, its elimination half-life

typically ranges from 12 to 15 hours but can vary. Metabolism primarily occurs via CYP2A4, resulting in several active metabolites, with excretion predominantly through feces.

Drug Interactions

As sirolimus is metabolized by CYP2A4 and serves as a substrate for the P-glycoprotein (P-GP) drug efflux pump, medications such as voriconazole, itraconazole, fluconazole, and erythromycin elevate its blood concentration. Conversely, inducers of CYP3A4 decrease sirolimus blood levels. Cyclosporine enhances sirolimus bioavailability, potentially due to P-GP inhibition and competition for CYP3A4. When administered four hours apart, the bioavailability of both drugs exceeds 30–40%, while simultaneous administration results in over 100% bioavailability. Combining tacrolimus and sirolimus leads to greater renal toxicity compared to co-administration of cyclosporine and sirolimus.

Toxicity

Serious adverse effects of sirolimus may encompass allergic reactions, heightened susceptibility to infections, and the development of lymphoma. Nephrotoxicity is not a prominent concern unless the drug is combined with cyclosporine or tacrolimus. Less severe side effects associated with sirolimus administration include gastrointestinal discomfort, elevated cholesterol and triglyceride levels, acne, insomnia, tremors, muscle weakness or soreness, fluid retention or swelling, anemia, leukopenia, thrombocytopenia, and potential impairment of wound healing.

Clinical Uses

Sirolimus finds application in tissue transplantation, offering a significant advantage over calcineurin inhibitors by virtue of its non-nephrotoxic nature. Long-term administration of calcineurin inhibitors in transplant patients, which may lead to chronic renal failure, can be mitigated with sirolimus. Achieving steroid-free immunosuppression is possible through the use of sirolimus alone or in conjunction with mycophenolate mofetil, cyclosporine, or tacrolimus. However, due to the potential side effect of impaired wound healing, some transplant centers reserve sirolimus for administration several weeks post-surgery.

Additionally, sirolimus has been utilized in the fabrication of sirolimus-eluting stents employed for treating obstructive coronary arteries. The rationale behind incorporating sirolimus in these stents lies in its antiproliferative properties. Moreover, sirolimus has garnered attention for

cancer therapy owing to its antiproliferative effects. Animal studies have revealed promising results regarding sirolimus's potential in cancer treatment, particularly in combination with doxorubicin, showing remission in AKT-positive lymphomas.

Everolimus (Zortress)

Everolimus, a derivative of sirolimus, shares a similar mechanism of action but exhibits greater hydrophilicity and a distinct pharmacokinetic profile compared to sirolimus. It boasts a shorter half-life and demonstrates efficacy in conditions such as cardiac allograft vasculopathy and posttransplant lymphoproliferative disorders. Binding to its cytoplasmic receptor FKBP12, everolimus forms a complex that interacts with mTORC1, leading to the inhibition of downstream signaling pathways. By targeting mammalian target of rapamycin (mTOR), everolimus serves in preventing organ rejection (Zortress) and treating certain cancers (Afinitor). Its action via the mTORC1 protein complex does not affect mTORC2, allowing for the activation of AKT through the inhibition of the negative feedback loop mediated by mTORC1. Consequently, mRNA coding for proteins involved in the cell cycle is inhibited, curbing the growth and proliferation of target cells.

Widely employed in kidney, heart, liver, and other transplants, everolimus aids in reducing chronic allograft vasculopathy, particularly in cardiac transplantation, although its use in this context has sparked some controversy due to an associated increase in mortality rates. Additionally, it functions as an immunosuppressant to prevent restenosis in drug-eluting coronary stents, being marketed as the Promus everolimus-eluting coronary stent system.

Typically administered alongside cyclosporine, everolimus requires close monitoring for cyclosporine-induced nephrotoxicity, which may vary depending on the dosage, along with the potential for hyperlipidemia. Common adverse effects of everolimus encompass an elevated risk of infection and specific cancers, rash, edema, leukopenia, facial, arm, and leg swelling, chest pain, breathing and swallowing difficulties, flu-like symptoms, mouth sores, tingling in extremities, gastrointestinal disturbances, weight changes, and urinary difficulties. Within a 30-day period, everolimus may elevate the risk of blood clot formation, posing a threat to transplanted kidneys. Moreover, being a substrate for CYP3A4, everolimus is susceptible to alterations in blood levels and clearance when interacting with drugs affecting this enzyme system, including ACE inhibitors, statins, fibrates, azole antifungal agents, macrolide antibiotics, digoxin, diltiazem, verapamil, carbamazepine, dexamethasone, phenobarbital, rifampin, and protease inhibitors.

Sphingosine-1-Phosphate Receptor (S1P-R) Modulators

Fingolimod (Gilenya)

Fingolimod, a synthetic analogue of myriocin (ISP-1), is derived from the culture filtrates of the fungus Isaria sinclairii. Following phosphorylation, it exhibits high-affinity binding to G-protein-linked sphingosine-1-phosphate receptor 1 (S1P1) on lymphocytes and thymocytes, resulting in receptor internalization. Consequently, lymphocytes are unable to respond to serum lipid sphingosine-1-phosphate (S1P), impeding their egress from lymphoid organs and circulation into graft sites and peripheral inflammatory tissues. This novel immunosuppressive mechanism induces a reduction in peripheral blood lymphocyte count through emigration of blood lymphocytes to secondary lymphoid tissue and apoptotic T-cell death.

Moreover, fingolimod modulates lymphocyte chemotactic responses, accelerating T-cell trafficking in secondary lymphatic tissue. It also preserves vascular integrity by enhancing endothelial barrier function and adherens junction assembly. Notably, fingolimod does not affect the activation, proliferation, or effector function of T and B cells. It exhibits efficacy in preventing acute graft rejection comparable to mycophenolate mofetil in new renal transplant patients.

Pharmacokinetically, fingolimod is characterized by a prolonged absorption phase, a large distribution volume, and a long elimination half-life. Its most common side effect is asymptomatic transient bradycardia, unlike other immunosuppressive agents associated with hyperlipidemia, diabetes mellitus, and renal toxicity.

Fingolimod marks the first oral drug approved for multiple sclerosis to reduce relapse and delay disability progression in relapsing patients. Multiple sclerosis, an inflammatory demyelinating and neurodegenerative disease of the central nervous system, involves immune system dysregulation, characterized by plaque production and immune cell infiltration, particularly T cells, B lymphocytes, plasma cells, and macrophages. Fingolimod sequesters lymphocytes in secondary lymphoid organs, preventing their migration to the central nervous system, thus mitigating autoreactive responses and consequent damage.

Furthermore, fingolimod is under evaluation for heart failure and arrhythmias treatment. It activates Pak1, a regulator of cardiac cell growth, motility, and survival, thereby preventing cardiac hypertrophy and arrhythmias in animal models.

Belatacept (Nulojix)

Belatacept, a product of recombinant DNA technology, is constructed by fusing the Fc region of the antibody IgG1 with the extracellular domain of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. CTLA-4, found on the surface of helper T cells and regulatory T cells, transmits inhibitory signals. Structurally similar to CD28, CTLA-4 binds to CD80/86 on antigen-presenting cells, molecules responsible for T cell activation. CTLA-4 disrupts this process through potential mechanisms such as removing CD80 and CD86 from the cell surface or modulating cell motility and signaling via PI3-kinase.

Belatacept binds to CD80/86, inhibiting the second signal of helper T cell activation and inducing their deactivation. This selective blockade of T cell activation aims to prolong graft survival while minimizing the toxicity associated with conventional immunosuppressive regimens like calcineurin inhibitors.

Belatacept, in combination with basiliximab, mycophenolate mofetil (MMF), and corticosteroids, is indicated for preventing organ rejection in adult kidney transplant recipients, specifically those who are Epstein–Barr virus (EBV) seropositive. However, recipients of belatacept are at an increased risk of developing posttransplant lymphoproliferative disorder (PTLD), primarily involving the central nervous system (CNS). Other adverse effects include cytomegalovirus (CMV) infection, T-cell-depleting therapy, progressive multifocal leukoencephalopathy (PML), and heightened susceptibility to various infections, including bacterial, viral, fungal, and protozoal infections, opportunistic infections, tuberculosis, and malignancies.

Abatacept (Orencia)

Abatacept, developed through recombinant DNA technology, comprises the fused Fc region of antibody IgG1 and the extracellular domain of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). This protein, present on helper T cells, transmits inhibitory signals and shares structural similarities with CD28. Both CTLA-4 and CD28 bind to CD80/86 on antigen-presenting cells, molecules crucial for T cell activation.

Differing from belatacept by only two amino acids, abatacept binds to CD80/86, hindering the second signal required for helper T cell activation and potentially deactivating them. By impeding this signal, abatacept aims to prevent T cell activation or induce an anergic state. It

is utilized in adults and children (at least 6 years old) with rheumatoid arthritis unresponsive to other disease-modifying antirheumatic drugs. Abatacept is effective in slowing structural damage progression and alleviating rheumatoid arthritis symptoms.

However, caution is advised against combining abatacept with Kineret and other drugs targeting TNF-α. Adverse effects may include fatigue, sore throat, dry cough, difficulty breathing, wheezing, fever, chills, night sweats, flu-like symptoms, weight loss, skin irritation, and gastrointestinal discomfort.

Mycophenolate Mofetil (Cellcept)

Mycophenolic acid (MPA) was first identified in cultures of Penicillium spp. in 1896 and subsequently purified in 1913. Initially investigated for its antifungal and antibacterial properties, it later garnered attention for its potential antitumor effects. It wasn't until later years that its immunosuppressive properties were recognized. Following extensive research and development, mycophenolate mofetil, an ester prodrug of mycophenolic acid, was formulated. Approved by the United States Food and Drug Administration in 1995 for preventing acute renal allograft rejection and in 1998 for heart transplant recipients, mycophenolate mofetil has since become a cornerstone of immunosuppressive therapy.

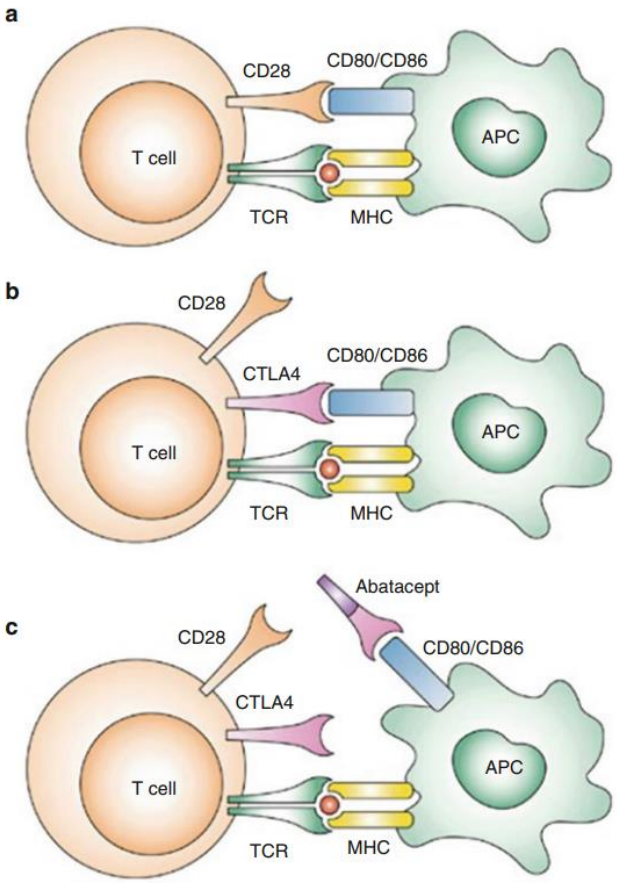

Figure 5. Abatacept's mechanism of action involves antagonizing the B7 ligands, CD80 (B7-1) and CD86 (B7-2), which are found on antigen-presenting cells. These ligands are crucial for initiating the second signal necessary for T cell activation. Upon T cell activation, CTLA-4 expression increases. CTLA-4 binds to CD80/86, dampening T cell activation. Abatacept disrupts the interaction between CD80/CD86 and CD28.

Mechanism of Action

Mycophenolate mofetil functions as a selective cytotoxic agent targeting B and T lymphocytes by inhibiting the production of guanosine nucleotides necessary for DNA synthesis. Unlike other blood marrow elements and parenchymal cells, which utilize salvage pathways, B and T lymphocytes primarily rely on de novo synthesis for purine biosynthesis. Mycophenolate mofetil acts as a reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMP-DH), a key enzyme in de novo guanosine nucleotide synthesis. This inhibition leads to the suppression of lymphocyte proliferation and antibody synthesis by B cells. Additionally, mycophenolate mofetil may impede the recruitment of leukocytes to inflammatory sites by altering the glycoproteins involved in lymphocyte adhesion and migration, which is particularly beneficial in organ transplantation. Notably, mycophenolate mofetil does not specifically impact the synthesis of cytokines, their receptors, or cytokine receptor-dependent signaling pathways.

Absorption, Distribution, and Excretion

After oral intake, mycophenolate mofetil is swiftly absorbed, boasting a high oral bioavailability of 94%. Once ingested, it undergoes metabolism by esterases, converting into its active form, mycophenolic acid (MPA). The enterohepatic recirculation process significantly influences the serum concentrations of MPA. Subsequently, the active metabolite undergoes further metabolism by glucuronyl transferase before being predominantly excreted (90%) in the urine as MPA glucuronide, facilitated by the organic anion transport system in the proximal tubule. A minor portion is eliminated via feces.

Drug Interactions

When mycophenolate mofetil is taken with antacids, its absorption decreases. Cholestyramine, by binding to mycophenolate mofetil glucuronide (MPAG) in the intestine, disrupts the enterohepatic recirculation of the drug, significantly reducing plasma MPA concentration. Interestingly, the coadministration of mycophenolate mofetil with tacrolimus, compared to cyclosporine, enhances its bioavailability. Additionally, antibiotics such as fluoroquinolones and metronidazole diminish the bioavailability of MPA.

Toxicity

Side effects associated with mycophenolate mofetil comprise diarrhea, abdominal pain, constipation, nausea/vomiting, acne, dyspnea, cough, peripheral edema, heightened susceptibility to infections, drug-induced fever, dizziness, headaches, leukopenia, and anemia.

Clinical Uses

Mycophenolate mofetil is employed in tissue transplantation alongside tacrolimus, cyclosporine, or sirolimus in combination with glucocorticoids. It is the most frequently utilized cytotoxic drug, either administered during transplantation or after the onset of acute rejection. Mycophenolate mofetil serves as a prophylactic measure and is not suitable for addressing chronic rejection or ongoing acute rejection.

Azathioprine

Initially developed for chemotherapy, azathioprine is now primarily employed as an immunosuppressive agent, with its use as an antineoplastic drug being rare. It was first introduced as an immunosuppressant by Roy Calne, a pioneering figure in tissue transplantation from Britain. Azathioprine finds application in preventing rejection post-tissue transplantation, serving as a substitute for 6-mercaptopurine due to its lower toxicity. Apart from tissue transplants, it is also utilized in conditions like rheumatoid arthritis and Crohn's disease. Azathioprine acts as a prodrug, undergoing conversion in the body to its active metabolites: 6-mercaptopurine and 6-thioinosinic acid. Prior to the discovery of cyclosporine, the standard regimen for preventing rejection post-tissue transplantation involved azathioprine in combination with steroids.

Mechanism of Action

Azathioprine works by inhibiting purine synthesis, a crucial process for cell proliferation, particularly in immunocompetent cells. Upon reaction with glutathione, azathioprine is converted into 6-mercaptopurine, which in turn produces additional metabolites that hinder de novo purine synthesis. This inhibition occurs through the synthesis of 6-thio IMP, 6-thio GMP, and 6-thio GTP. Eventually, cell proliferation is suppressed when 6-thio GTP is incorporated into the host DNA.

Absorption, Distribution, and Excretion

Azathioprine is absorbed orally, reaching peak blood levels within 1–2 hours. While the parent drug has a short half-life of 10 minutes, its metabolite 6-mercaptopurine persists longer with a half-life of nearly 1 hour, and certain metabolites have even longer half-lives. Both the prodrug and its active metabolites bind to plasma proteins with low affinity and are eliminated from tissues through oxidation or methylation processes.

Drug Interactions

The concurrent use of purine analogues like allopurinol and azathioprine is discouraged. Thiopurine S-methyltransferase (TPMT) enzyme activity inhibits 6-mercaptopurine. Genetic variations in TPMT can elevate azathioprine toxicity, making it beneficial to monitor serum TPMT levels to prevent adverse effects. When combined with angiotensin-converting enzyme inhibitors or other myelosuppressive drugs, azathioprine may lead to leukopenia, anemia, and thrombocytopenia.

Toxic Effects

Azathioprine is classified as a human carcinogen, although some studies have yielded inconclusive results. Individuals previously treated with alkylating agents may face an increased cancer risk with azathioprine therapy. It is not associated with fetal malformations. Short-term adverse effects of azathioprine include myelosuppression (anemia, leukopenia, and thrombocytopenia), heightened cancer and infection risks, gastrointestinal disturbances, alopecia, and hepatotoxicity.

Clinical Uses

Azathioprine is prescribed for patients unresponsive to calcineurin inhibitors, sirolimus, and glucocorticoids. Prophylactic therapy typically involves daily doses of 3–10 mg/kg administered 1 or 2 days pre-renal transplantation or on the surgery day. Mycophenolate mofetil is increasingly preferred over azathioprine for tissue transplantation due to its lower myelotoxicity and reduced risk of opportunistic infections.

Cyclophosphamide

Cyclophosphamide disrupts DNA synthesis and cell proliferation mechanisms by alkylating DNA in both proliferating and nonproliferating cells. Its immunosuppressive effects mirror its

antineoplastic actions. While it affects both B and T cells, B cells experience greater toxicity due to slower recovery. Cyclophosphamide has varied effects on T-cell-mediated immunity, occasionally augmenting responses, but overall, it tends to inhibit them. In most preparative regimens preceding allogeneic bone marrow transplantation, cyclophosphamide is combined with another cytotoxic agent, often with antithymocyte globulin or total lymphoid irradiation, to mitigate rejection risks. Additional cytotoxic agents used alongside cyclophosphamide may include busulfan, fludarabine, or treosulfan. Combinations like fludarabine and cyclophosphamide, with or without antithymocyte globulin, are also employed in preparative regimens for cord blood transplantation and allogeneic stem cell transplantation to minimize rejection risks.

Glucocorticoids

Cortisone was the inaugural immunosuppressive agent identified. Glucocorticoids, synthesized by the adrenal cortex, consist of 21 carbon atoms. While they exert diverse biological functions impacting various tissues and organ systems, this discussion will focus solely on their immunosuppressive effects. Since the 1960s, glucocorticoids have been integral in tissue transplantation to forestall rejection. Presently, they are administered alongside other immunosuppressive agents to avert rejection of transplanted tissue. Nevertheless, owing to their severe effects, there has been a shift towards promptly withdrawing glucocorticoids post-tissue transplantation and transitioning to steroid-free immunosuppressive regimens.

Mechanism of Action

Glucocorticoids exert their inhibitory effects on acquired or cell-mediated immunity by targeting genes responsible for the synthesis of various cytokines. They suppress the production of a wide array of cytokines, encompassing IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, and INF-γ. Among these, the inhibition of IL-2 by corticosteroids is particularly pivotal in immunosuppression, leading to the curtailment of T-cell proliferation and the activation of cytolytic T cells. Additionally, glucocorticoids modestly impact humoral immunity by impeding B-cell clonal expansion and antibody synthesis. These effects occur through the suppression of IL-2 and IL-2 receptor expression in B cells.

Glucocorticoids serve as potent inhibitors across all phases of the inflammatory process. This inhibition is achieved by inducing the synthesis of lipocortin (annexin-1). Lipocortin, upon binding to the cell membrane, obstructs the access of phospholipase A2 to its substrate arachidonic acid, thereby reducing eicosanoid production. This effect is further accentuated by the suppression of cyclooxygenase activity. Moreover, glucocorticoids promote the extracellular release of lipocortin 1, which binds to various leukocyte membrane receptors, leading to the inhibition of inflammation. Processes such as adhesion, chemotaxis, respiratory burst, and phagocytosis are among those affected by this mechanism. Furthermore, glucocorticoids inhibit the release of diverse inflammatory mediators from mononuclear and polymorphonuclear phagocytes. By forming complexes with glucocorticoid receptors, they impede the activation of NF-κB, consequently increasing the apoptosis of activated cells. In summary, glucocorticoids induce a rapid, transient reduction in the circulating peripheral blood lymphocyte count.

Absorption, Distribution, and Excretion

Glucocorticoids can be administered via oral, intravenous, or intramuscular routes, and they are absorbed locally at the site of administration, such as the skin, eye, and respiratory tract. Prolonged local exposure may lead to systemic effects over time. Most corticosteroids bind to two plasma proteins, transcortin (corticosteroid-binding globulin, CBG), and albumin. At higher doses, when all plasma-binding protein sites are saturated, corticosteroids may exist in an unbound form. Metabolism of glucocorticoids results in the formation of water-soluble derivatives. This process of reduction can occur both in the liver and elsewhere in the body, leading to the formation of inactive compounds, with subsequent hepatic reduction being the predominant pathway. Enzymatic reactions convert glucocorticoids into either glucuronide or sulfate forms, which are then excreted in urine.

Side Effects

The common side effects of glucocorticoids used for preventing transplant rejection include hyperglycemia, increased susceptibility to infections, impaired wound healing, bone density reduction, ulcers, muscle weakness, fluid retention, skin irritation, excessive facial hair growth, and facial swelling. In children, their use may also lead to growth retardation.

Clinical Uses

Glucocorticoids are employed alongside other immunosuppressive agents to prevent transplant rejection. In cases of acute transplant rejection, high doses of intravenous methylprednisolone are often administered. They are effective in suppressing allergic reactions to other immunosuppressive agents and counteracting the effects of cytokines associated with the use of anti-CD3. Glucocorticoids are also utilized in bone marrow transplantation to prevent graft-versus-host disease. Additionally, they find application in treating autoimmune diseases, asthma, psoriasis, dermatomyositis, and inflammatory eye conditions.

Polyclonal antibodies

Antithymocyte and antilymphocyte globulins are derived from animals and targeted against human T cells, finding utility in treating acute rejection in organ transplantation and aplastic anemia. These polyclonal antibodies contain cytotoxic elements that bind to various antigenic markers on T lymphocytes, leading to their elimination from circulation. Additionally, they impede lymphocyte function by interacting with critical regulatory molecules on the lymphocyte cell surface.

In the United States, two antithymocyte globulins are sanctioned for use: Atgam, an equine immunoglobulin, and thymoglobulin, a rabbit immunoglobulin. Employed in conjunction with other immunosuppressive agents, antithymocyte globulins are utilized to address acute renal transplant rejection. They may serve as an initial alternative to nephrotoxic calcineurin inhibitors for renal transplant patients, safeguarding the transplanted tissue. Typically administered at a dose of 1.5 mg/kg daily for 1–2 weeks for acute renal graft rejection, they have also found application in liver transplantation. In the United States, antithymocyte globulin is administered during transplantation to forestall graft-versus-host disease, whereas in Europe, it is preferred for steroid-resistant acute rejection. However, both formulations are also utilized off-label before or during kidney transplants. Side effects may include fever, chills, serum sickness, leukopenia, thrombocytopenia, heightened infection risk, and malignancies, particularly when combined with other immunosuppressive drugs, prompting debate on their use and whether the benefits outweigh the risks.

Antilymphocyte globulins (ALG), derived mainly from equine sources and lacking a brand name, are less frequently employed than antithymocyte globulins. Both carry the risk of precipitating post-transplant lymphoproliferative disorder and cytokine-release syndrome. Post-transplant lymphoproliferative disorder involves B-cell proliferation due to therapeutic immunosuppression, potentially triggered by Epstein–Barr virus infection and potentially treated with cyclosporine or tacrolimus. Cytokine-release syndrome arises when antibodies to T cells activate them, leading to systemic inflammatory response syndrome-like symptoms such as hypotension, fever, and shivering. Treatment may involve corticosteroids, antihistamines, and acetaminophen.

Rho (D) immune globulin stands out as a highly specific and effective immunosuppressive intervention. These IgG antibodies boast elevated Rh (D)-specific titers. Administered to Rh-negative mothers post-delivery of an Rh-positive baby, Rho (D) immune globulin thwarts the development of antibodies against Rh-positive cells, averting hemolytic disease of the newborn and erythroblastosis fetalis. Its intramuscular administration yields a half-life of approximately 3–4 weeks, with potential side effects encompassing injection site discomfort and mild fever, while extremely rare occurrences of anaphylactic shock have been reported.

Monoclonal Antibodies

Monoclonal antibodies utilized as immunosuppressants in tissue transplantation comprise muromonab-CD3, daclizumab, and basiliximab. Muromonab-CD3 targets a specific site on the CD3 receptor, impeding the T cell receptor's interaction with antigens and suppressing CD3 receptor-dependent signal transduction pathways, thereby inducing immune suppression. Conversely, daclizumab and basiliximab are monoclonal antibodies designed to target IL-2 receptors, thus hindering IL-2-dependent responses following tissue transplantation, ultimately leading to immune suppression.

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