

PHARMACOLOGIC IMMUNOSUPPRESSION

Neal R. Barshes, Sarah E. Goodpastor, and John A. Goss

Michael E. DeBakey Department of Surgery, Baylor College of Medicine, 6550 Fannin. Suite 1628, Houston, TX 77030

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Antimetabolites
 - 3.1. Azathioprine
 - 3.2. Mycophenolate mofetil (MMF)
4. Corticosteroids
5. Calcineurin Inhibitors
 - 5.1. Cyclosporine
 - 5.2. Tacrolimus
- 5.3. Sirolimus
6. Polyclonal Antibody Therapy
7. Monoclonal Antibody Therapy
 - 7.1. OKT3
 - 7.2. Daclizumab and Basiliximab
8. Pharmacologic Agents in Development: FTY720
9. Summary
10. References

1. ABSTRACT

Clinical organ transplantation only became a viable treatment option after the advent of effective pharmacologic immunosuppression. Azathioprine and steroids were among the first drugs available for pharmacologic immunosuppression allowed for the first long-term successes in kidney and liver transplantation, though survivors experienced significant adverse effects of the immunosuppression. Azathioprine is an antimetabolite which inhibits the *de novo* and salvage pathways of purine synthesis. This results in lymphocyte suppression but also toxicity to bone marrow, gastrointestinal tract, and liver. Mycophenolate mofetil (MMF), another antimetabolite drug, inhibits only the *de novo* purine synthesis pathway. Corticosteroids cause immunosuppression mainly by sequestration of CD 4+ T-lymphocytes in the reticuloendothelial system and by inhibiting the transcription of cytokines. Corticosteroids have adverse effects on virtually every system in the body, producing many dose-limiting problems such as osteoporosis, obesity and glucose intolerance.

The introduction of cyclosporine in 1983 allowed for further improvements in graft survival, and the incidence of acute rejection decreased. Cyclosporine and the more recently-introduced tacrolimus compose the class of immunosuppressive agents called calcineurin inhibitors. By binding calcineurin and preventing its translocation into the nucleus these drugs prevent transcription and subsequent secretion of IL-2. These drugs produce varying degrees of nephrotoxicity, neurotoxicity and glucose intolerance. Rapamycin also inhibits IL-2 expression, though by interaction with the mammalian Target of Rapamycin (mTOR) protein.

The use of antibody to produce immunosuppression began with polyclonal sera developed in animals such as horses or goats. The mechanism by which polyclonal sera causes immunosuppression is not well understood, though cell-mediated cytotoxicity of lymphocytes in the circulation may be one major effect. In contrast, the monoclonal antibody OKT3 is specific for the T-cell receptor (TCR) / CD3 complex, thus preventing activation of T-lymphocytes. Most recently, human and chimeric murine monoclonal antibodies daclizumab and basiliximab have provided effective induction therapy with virtually no adverse effects.

While the improved efficacy and decreased adverse effects immunosuppressive agents account for much of the progress in the field of transplantation, current immunosuppression medications not perfect. Ideally, medications would inducing graft tolerance while avoiding generalized immunosuppression and non-immunologic adverse effects. Future research will likely focus on molecular- and gene-level mechanisms to achieve this goal.

2. INTRODUCTION

Two developments that occurred in the twentieth century allowed solid organ transplantation to become a reality: first, the development of the technique of vascular anastomoses by Dr. Alexis Carrel; and second, the development of effective pharmacologic immunosuppression to prevent rejection of the allograft. Between World War I and II many renal transplants had been attempted; virtually all had failed. By the end of World War II, however, Medewar's experiments with skin grafts had illustrated the

role that the immune system played in the destruction of allografts. With the observation that the radiation-exposed Japanese survivors of Nagasaki and Hiroshima were immunosuppressed, whole-body irradiation became the first form of immunosuppression used for transplantation. When azathioprine was shown to be more effective than whole-body irradiation, drug therapy became the mainstay for inducing immunosuppression (Stark).

The ideal immunosuppressive agent would induce tolerance of the graft without causing generalized immune suppression, leaving the host susceptible to opportunistic infections. It would have few non-immunological adverse effects and would have a predictable pharmacokinetic profile. Obviously, the ideal immunosuppressive agent does not yet exist, though with time, immunosuppressive therapies become more effective in preventing rejection while causing fewer adverse effects.

There are three “regimens” of immunosuppressive therapy: induction, maintenance, and anti-rejection. Induction therapy describes the combination of drugs given immediately after the transplant with the aim of preventing acute rejection. Maintenance immunosuppression consists of immunosuppression drugs used to prevent acute and chronic rejection. Anti-rejection therapy is a drug or combination of drugs given to treat an ongoing episode of acute rejection.

3. ANTIMETABOLITES

3.1. Azathioprine

Azathioprine (Imuran®, made by Glaxo-Wellcome) was among the first immunosuppressive drugs used in organ transplantation. Hitchings and Elion, in the Tuckahoe Laboratories, first developed 6-mercaptopurine (6-MP, a purine analog and parent drug of azathioprine) for use as a chemotherapeutic agent. In 1959 Schwartz and Dameshek, working at Tufts Medical School in Boston, noted that 6-mercaptopurine prevented rabbits from forming antibody to bovine albumin. The following year Sir Roy Calne at Cambridge University showed increased renal allograft survival in dogs. Hume and Zukoski had similar observations that same year. Azathioprine was compared to whole body irradiation by Murray at the Peter Bent Brigham hospital and shown to result in better outcomes. Further studies ultimately showed that a combination of azathioprine and steroids yielded the best outcomes the field of transplantation had seen thus far. Outcomes before the use of azathioprine and steroids were dismal; after their introduction five year patient survival increased to 50%. From the early 1960s until cyclosporine was introduced in 1983, the combination of azathioprine and steroids was considered “conventional” immunosuppression and the standard regimen for maintenance immunosuppression (1).

Azathioprine is an imidazole derivative of 6-mercaptopurine. After ingestion and absorption of the drug by the gastrointestinal tract, azathioprine is converted to 6-mercaptopurine by the glutathione-S-transferase in erythrocytes. 6-mercaptopurine is then metabolized via one

of three pathways: to 6-thioinosinic acid and 6-thioguanine acid via hypoxanthine-guanine-phosphoribosyl transferase (HGPRT); to thiouric acid via xanthine oxidase (XO); and to 6-methyl-mercaptopurine via thiopurine methyltransferase (TPMT). 6-thioinosinic acid and 6-thioguanine acid are active metabolites which interfere with metabolism of inosine-monophosphate (IMP) to adenosine-monophosphate (AMP) and adenosine triphosphate (ATP) in RNA and DNA synthesis in the salvage pathway. Thus, it interferes with purine synthesis and inhibits *de novo* purine synthesis. This results in a suppression of proliferating B- and T-lymphocytes. It also has some anti-inflammatory action (1).

The most common adverse effect of azathioprine is bone marrow depression -- mainly leukopenia, but also macrocytic anemia and thrombocytopenia. Dose reduction should be considered when leukocyte counts fall below 4000 cells/mL. Gastrointestinal toxicity (specifically, nausea, vomiting and diarrhea) occurs often. Hepatotoxicity occurs by an unknown mechanism, though it is now thought that some of the hepatotoxicity attributed to azathioprine in past studies may have in fact been undiagnosed viral hepatitis. The hepatotoxicity is often manifest as an increase in liver enzymes, but toxicity occurs even at azathioprine doses too low to cause an elevation in these enzymes. Other common adverse effects include skin rashes and fever.

The typical dose of azathioprine is 2-5 mg/kg/day when used in combination with steroids for immunosuppression. After the addition of cyclosporine to “conventional” immunosuppression the dose was often lowered to 1-2 mg/kg/day. The dose of azathioprine should be reduced to 25-33% of normal for patients taking allopurinol, a drug that inhibits xanthine oxidase and thereby inhibits inactivation of azathioprine (see above). Angiotensin converting enzyme-inhibitors (ACE-Is) increase the myelosuppression associated with azathioprine by an unknown mechanism.

3.2. Mycophenolate mofetil (MMF)

Mycophenolate mofetil (MMF, or CellCept® produced by Roche Laboratories) is the semisynthetic morpholinoethyl ester prodrug of mycophenolic acid (MPA). MPA was first isolated from the mold *Penicillium glaucum* in 1898 (Katzung). While being investigated as a drug for psoriasis in the 1970s it was noted to have the adverse effects of myelosuppression and diarrhea. It was not until the 1990s that MPA was studied as a potential immunosuppressant (Pirsch, Neto).

After ingestion MMF is hydrolyzed to MPA in the stomach and small intestine. A first peak in serum drug level occurs about one hour after ingestion; a second peak occurs between six and twelve hours after ingestion and is attributed to enterohepatic cycling. A few drug-drug interactions are notable: bioavailability and serum trough concentrations of MPA are decreased by cyclosporine. Tacrolimus increases the area-under-the-curve and maximal concentrations of MPA. Finally, MMF should be given one hour before any antacids, as these may decrease absorption.

Pharmacologic Immunosuppression

The great majority of serum MPA is albumin-bound; only the 1.25% that remains free in the plasma is active. The half-life of the drug is about 17 hours. It is metabolized to mycophenolic acid glucuronide (MPAG) before excretion. Approximately 96% is excreted in the urine and the remainder in the urine.

The *de novo* purine synthesis pathway is one of two pathways for the synthesis of nucleic acids for incorporation into DNA and RNA. It is the exclusive purine synthesis pathway used in B- and T-lymphocytes, whereas other cells may still rely on the alternate *salvage* pathway. Adenosine triphosphate combines with ribose-5-phosphate to form 5-phosphoribosyl-1-pyrophosphate (PRPP) in a reaction mediated by PRPP synthetase. PRPP is then converted to inosine monophosphate (IMP), which is converted to guanosine monophosphate (GMP) in a reaction catalyzed by inosine monophosphate dehydrogenase (IMPDH). Further conversions ultimately result in deoxyguanylic acid (dGMP), which is incorporated into DNA chains.

MPA acts as a highly selective and reversible inhibitor of IMPDH, thus inhibiting the conversion of IMP to GMP. Because MMF inhibits only this *de novo* pathway, it is relatively selective for actively replicating lymphocytes. Of the two isoforms of IMPDH, MPA has a four to five times higher affinity for isoform II, the predominant isoform in the lymphocyte, further enhancing selectivity for lymphocytes. Unlike azathioprine, MMF is not a nucleotide analogue and thus will not produce the possible mutagenic effects such as inhibition of DNA repair enzymes (2).

The most common adverse effect of mycophenolate mofetil is gastrointestinal toxicity, producing nausea, vomiting, diarrhea and abdominal pain. Diarrhea is especially common with the combination of cyclosporine and MMF. Bone marrow suppression also occurs. MMF is a potential teratogen and therefore should not be used in pregnant women (3); it also decreases effectiveness of oral contraceptives.

MMF has been proven more effective maintenance therapy than azathioprine, yet it is more expensive. The increased cost of MMF is offset by the decreased expenses in treating episodes of acute rejection. Some authors suggest, though, that azathioprine can be substituted for MMF in a stable transplant patient 3-6 months after the transplant if they have had no episodes of acute rejection.

4. CORTICOSTEROIDS

The potential of corticosteroids as an immunosuppressive agent was first demonstrated in a canine model of allograft rejection by Dempster in 1953. Medewar and Morgan independently showed that the local or systemic administration of steroids significantly lengthened the lifetime of skin grafts in rabbits (4). Starzl, then at the Veteran's Administration Hospital in Denver, Colorado was the first to use the combination of azathioprine and corticosteroids in renal transplant patients.

He presented survival results in 1963 that were a great improvement over previous immunosuppression regimens (4). Hume, at the Peter Bent Brigham Hospital, independently presented results using corticosteroids during that same year. This combination became regarded as "conventional" immunosuppression and was the mainstay of pharmacologic immunosuppression until the introduction of cyclosporine in 1983.

Corticosteroids have two main immunosuppressive effects on the immune system: the sequestration of CD4+ T-lymphocytes in the reticuloendothelial system (RES); and inhibition of both proliferation and function of lymphocytes via inhibition of lymphokines and cytokines. Upon administration, the hydrophilic corticosteroid molecule diffuses into the cytoplasm. In the cytoplasm corticosteroids displace heat-shock proteins (HSPs) and forming a complex with heat shock protein-receptor. Corticosteroids bind the HSP-receptor then, in the nucleus, bind to DNA sites called corticosteroid response elements (GREs). The result is an inhibition in transcription of lymphokine and cytokine genes, especially IL-1 and IL-6.

Other immunosuppressive effects of steroids include:

- inhibition of delayed hypersensitivity.
- increase in endonucleases, leading to an increased rate of apoptosis of lymphocytes.
- suppression of display of cell-cell adhesion molecules, thus inhibiting migration of leukocytes.
- inhibition of antigen processing and display.
- inhibition of production of inflammatory mediators, including leukotrienes, prostaglandins, histamine, bradykinins.
- Inhibition of the action of macrophage migration-inhibition factor (MIF).
- Inhibition expression of IL-2 and prevents interaction of IL-2 with T-cell receptors, thereby suppressing the activation of T lymphocytes.
- increased fractional catabolism and decreased synthesis of IgG.

The half-life of cortisol in the circulation is approximately 60-90 minutes. Some drugs, including phenytoin, phenobarbital and rifampin decrease the half-life of corticosteroids by induction of the hepatic P450 enzyme system (4). Native cortisol is normally 75% bound to cortisol-binding globulin (CBG), 20% free, and 5% albumin-bound, whereas synthetic corticosteroids (ex. dexamethasone) are largely albumin-bound (5).

There are many corticosteroids available, varying greatly in their potency, mineralocorticoid activity, and expense. The three most often used are methylprednisolone (Solumendrol®), prednisolone and prednisone.

There are many adverse effects of corticosteroids, especially with high-dose regimens. These effects are seen after as little as two weeks. Among the most common adverse effects is the inhibition of bone formation and acceleration of bone resorption, often leading to

osteoporosis. The etiology has not been completely elucidated, but it is known that bone metabolism is affected in the following ways: stimulation of osteoclasts; decreased rate of production of osteoblasts from progenitor cells; decreased gastrointestinal reabsorption of calcium; increased urinary excretion of calcium; and inhibition of hydroxylation of vitamin D in the liver. A secondary increase in parathyroid hormone (PTH) levels occur, further accelerating bone reabsorption. Osteoporosis is often manifest as back pain with or without vertebral compression fractures. Avascular (or aseptic) necrosis of bone, most often the femur or humerus) occurs less often (6).

Many skin changes occur with corticosteroid administration. Wounds healing is inhibited, secondary to inhibition of fibroblast activity. Striae occur in 50-70% of patients as a result of a loss of tissue collagen and an increase in subcutaneous fat deposition. Acanthosis nigricans occurs in the intertriginous areas (submammary skin, axillae, groin areas). "Steroid acne" refers to pustular or papular lesions on the face, chest and back that occur with increased frequency. Easy bruisability, facial telangiectasias, and facial plethora are also attributed to excess corticosteroids. Finally, fungal infections of the skin and mucous membranes are seen in increased frequency, including tinea versicolor, onychomycosis, and oral candidiasis (6).

Centripetal obesity and a redistribution of fat moves adipose tissue from the extremities to the face and upper back, producing the so-called "moon facies" and the "buffalo hump," respectively. Muscle wasting occurs, often causing proximal muscle weakness. A redistribution of hair occurs as well, with hair loss from the head and a growth of fine hair of the thighs, trunk and occasionally the face. Hirsutism occurs in up to 80% of female patients with high-dose corticosteroid therapy (6).

A form of diabetes often develops in which the hyperglycemia is somewhat resistant to insulin but rarely features ketoacidosis. The mechanisms by which steroids cause hyperglycemia include: increased hepatic glucose production via stimulation of gluconeogenesis; increased insulin resistance; affecting free fatty acid metabolism (7).

Excess corticosteroids appear to suppress the normal pulsatile production of gonadotropin-releasing hormone (GnRH), thereby suppressing luteinizing hormone (LH) and follicular-stimulating hormone (FSH) production. This results in infertility and irregular menstruation or amenorrhea in women and decreased testosterone in men, with subsequent decrease in libido, impotence and soft testes (6). Other hormonal effects include reduced secretion of thyroid-stimulating hormone (TSH) and growth hormone. These effects, the previously mentioned inhibition of bone formation and stimulation of bone resorption, as well as direct effects of corticosteroids on the growth plate explain the growth retardation that occurs in pediatric patients given corticosteroid therapy (6). However, pediatric patients with end-stage renal disease also experience significant growth retardation. In spite of

the growth retardation caused by steroids, a child's growth is usually improved after transplantation, with post-transplant growth being inversely related to the size of the child prior to transplantation. In particular, severely growth-retarded children less than one year of age at the time of transplantation experience "catch-up growth" (i.e. grow to near average size for their age), while patients older than six years of age experience neither an adolescent growth spurt nor accelerated growth post-transplant (8).

The white blood cell count is usually normal, though the percentage and total number of lymphocytes is often decreased. Corticosteroids may also increase the number of red cells and platelets. Serum electrolytes are usually normal (6).

Many neuropsychiatric changes occur. Appetite is increased, and insomnia is common. Corticosteroids lower the threshold for electrically-induced seizures in rats. Neuropsychiatric effects in humans are common and occur in a dose-related manner; these effects include anxiety, depression, emotional lability, delirium and mania as well as symptoms such as headache, tremor, ataxia, disorientation, visual hallucinations, and seizures (9). Pseudotumor cerebri occurs with increased frequency in persons with excess corticosteroid levels (3). Increased intraocular pressure and glaucoma sometimes occur; this may interfere with the drainage of the aqueous humor of the globe and lead to the development of posterior subcapsular cataracts (6).

Because of the multitude of adverse effects, many transplant centers have attempted to eliminate the use of corticosteroids from the regimen for maintenance immunosuppression. Maintenance regimens which avoid the use of steroids are referred to as steroid-free protocols; these regimens have been shown to have a lower incidence of hypertension, hypercholesterolemia and diabetes. There appears to be no increase in incidence of acute rejection in liver allograft recipients in whom steroids are withdrawn after induction therapy (10).

A common corticosteroid regimen to treat acute rejection consists of one or two 1-gram doses of intravenous methylprednisolone followed by a six day "steroid taper" (i.e. progressive decrease in steroid dose to avoid adrenal suppression associated with sudden withdrawal of steroids). "Steroid-resistant" episodes of acute rejection are defined as a recurrence of acute rejection within 30 days of receiving steroids for the treatment of acute rejection (10).

5. CALCINEURIN INHIBITORS

5.1. Cyclosporine

Cyclosporine is a hydrophobic cyclic endecapeptide first isolated from the Norwegian fungal species *Tolypocladium inflatum* in the 1970s. Among the first to study the effects of cyclosporine on murine allografts was Sandoz scientist Jean Borel. The first clinical use of cyclosporine came after Borel demonstrated his results to Sir Roy Calne, a transplant surgeon at Cambridge

Table 1. Drug-Drug Interactions Involving Cyclosporine

Increase CSA levels	Decrease CSA levels	Synergistic nephrotoxicity	Reduced clearance c CSA
Diltiazem	Rifampin	Gentamicin	Prednisone
Ketoconazole	Carbamazepine	Amphotericin b	Lovastatin
Nicardipine	Phenobarbital	Tobramycin	Digoxin
Erythromycin	Phenytoin	Ketoconazole	
Verapamil	Inh	Cimetidine	
Itraconazole	Rifabutin	Vancomycin	
Danazol	Nafcillin	Melphan	
Bromocriptine	Octreotide	Bactrim	
Fluconazole	Ticlopidine	Ranitidine	
Methylpredisone		Azapropazon	
Metoclopramide		Diclofenac	
Grapefruit juice		Naproxen	
Clarithromycin		Sulindac	
High-dose steroids			
Indinavir			
Nelfinavir			
Ritonavir			

(11). The first clinical trial to show its efficacy was completed in Canada in 1986 (12).

Cyclosporine binds to, among other protein, the group of proteins called cyclophilins. Cyclophilin A binds with cyclosporine with high affinity, forming the cyclosporine-cyclophilin complex. The complex then inhibits calcium-activated calcineurin, preventing dephosphorylation and subsequent activation of DNA-binding proteins including nuclear factor of activated T-cells-1 (NFAT-1). Translocation of NF-AT from the cytosol to the nucleus is required for the transcription of the IL-2 gene, but this translocation occurs only when NF-AT is dephosphorylated. Thus cyclosporine results in inhibition of transcription of IL-2. Several proto-oncogenes are also inhibited as well. The cell is arrested in G0 or G1 phase of the cell cycle. Signal transduction is halted, and production of cytotoxic T lymphocytes and of specific antibody is attenuated.

Cyclosporine may be administered by parenteral or enteral routes. There are two enteral formulations: a gel cap with corn oil (Sandimmune®, by Novartis) or a microemulsion (Neoral®, also by Novartis). Compared to Sandimmune, there is much less variability in uptake and peak serum levels, both between individuals and within a given individual with Neoral. Bioavailability is approximately 30% with Sandimmune and 40-60% with Neoral. Peak serum concentration is reached in about 2.5 hours with Sandimmune and 1.5 hours with Neoral. One meta-analysis of Neoral-treated and Sandimmune-treated patients suggested that Neoral therapy was associated with a lower incidence of rejection when initiated immediately after the transplant, while stable patients on Sandimmune therapy benefited from converting to Neoral (13). There are many drug-drug interactions which involve cyclosporine; see Table 1.

Some of the more common adverse effects of calcineurin inhibitors include nephrotoxicity, neurotoxicity and diabetogenicity. The mechanism of nephrotoxicity

caused by cyclosporine is not known with certainty, but may be due to alteration in production of renal prostaglandins and/or decreased renal blood flow and increased renal vascular resistance at the level of the glomerular afferent arteriole. Three types of neurotoxicities to renal allografts have been described: acute, subacute and chronic. Acute nephrotoxicity occurs immediately after transplantation and is associated with intravenous cyclosporine administration. Subacute nephrotoxicity occurs at approximately 2-3 weeks after the transplant and typically presents as azotemia. A biopsy of the allograft will distinguish this type from acute rejection. Chronic nephrotoxicity occurs as a slow, steady deterioration of renal function. Effects of chronic rejection may be additive (13). Hypertension and glycosuria are often associated with cyclosporine use and may be a result of nephropathy (13).

Symptoms of neurotoxicity range from headache and tremor to seizures, tremor, coma or gray-white matter changes within the brain. The effects are typically dose-related. Gastrointestinal effects include diarrhea, dyspepsia, bloating and abdominal pain; these effects are more common when cyclosporine is combined with mycophenolate mofetil (MMF, see below). The taste of the liquid form is unpleasant, and may cause nausea. Hepatotoxicity, manifest as an increase in liver enzymes, has been reported. This hepatotoxicity is not associated with any histological change, though cyclosporine is usually avoided in patients with abnormal pre-transplant liver function tests because depressed liver function may alter serum cyclosporine concentration; and because frank cirrhosis may result (13). Calcineurin inhibitors have a toxic effect on the beta-cells of the pancreatic islets, which may result in hyperglycemia. This effect also appears to be dose-related and reversible (13).

The metabolic problems attributed to cyclosporine include hyperkalemia, hyperuricemia, and hyperlipidemia. Hyperkalemia and hyperuricemia are likely due to nephrotoxicity; the hyperuricemia may sometimes cause episodes of gout. Hyperlipidemia (specifically,

Table 2. Drug-Drug Interactions Involving Tacrolimus

Increase FK506 levels	Decrease FK506 levels	Synergistic Nephrotoxicity
Diltiazem	Rifampin	Gentamicin
Ketoconazole	Carbamazepine	Amphotericin b
Nicardipine	Phenobarbital	Tobramycin
Erythromycin	Phenytoin	Ketoconazole
Verapamil	Rifabutin	Cimetidine
Itraconazole		Vancomycin
Danazol		Melphan
Bromocriptine		Bactrim
Fluconazole		Ranitidine
Methylpredisone		Azapropazon
Metoclopramide		Diclofenac
Grapefruit juice		
Clarithromycin		
Cyclosporine		
Cimetidine		

hypercholesterolemia and hypertriglyceridemia) due to cyclosporine has been shown to increase cardiovascular mortality. Cyclosporine may also cause hirsutism and gingival hyperplasia. Facial dysmorphism is seen in pediatric transplant patients given cyclosporine.

5.2. Tacrolimus

Tacrolimus, or FK-506 (Prograf® by Fujisawa Healthcare, Inc.) is a macrolide antibiotic first isolated from the fungal species *Streptomyces tsukubensis* in 1985 while screening compounds for potential immunosuppressive medicines. It was first used in clinical practice by Starzl in 1989, when it was shown that tacrolimus could reverse acute liver allograft rejection which was recalcitrant to maximal cyclosporine therapy (14). This was followed by a non-randomized trial in which tacrolimus was initiated in the immediate post-operative period and compared to cyclosporine, and ultimately a randomized trial.

It binds to the FK binding protein 12 (FKBP12), and this complex then interacts with calcineurin as does the cyclosporin-cyclophilin complex (see above) (13). In addition to inhibiting transcription of the IL-2 gene, tacrolimus interferes with transcription of IL-3, IL-4, interferon-gamma, tumor necrosis factor (TNF)-alpha, and granulocyte-macrophage colony-stimulating factor (GM-CSF).

Oral bioavailability of tacrolimus may vary between 5 and 67%, depending among other variables on the presence of bile or food in the intestine. Its potency is ten-fold higher in vivo compared to cyclosporine. Like cyclosporine, it undergoes extensive hepatic metabolism and conjugation. More than 95% is excreted in the bile. The therapeutic half-life of tacrolimus is 11 hours. Trough levels are used to monitor for toxicity and adequacy of treatment. Goal trough levels are 15-20ng/mL in the immediate post-transplant period, 8-10 in the first three months post-transplant, and 5-7ng/mL thereafter (10). Many of the drug-drug interactions involving tacrolimus affect bioavailability; see Table 2.

Tacrolimus has also been associated with a lower incidence of acute rejection when compared to cyclosporine; however, there is no difference in graft survival at three or five

years post-transplant. The adverse effects of tacrolimus are similar to those of cyclosporine – namely neurotoxicity, nephrotoxicity and diabetogenicity. These are dose-related toxicities. However, absent from the effects of tacrolimus are the adverse cosmetic effects associated with cyclosporine – hirsutism, gingival hyperplasia and gynecomastia. Tacrolimus may decrease cholesterol and LDL levels but have no effect on LDL and triglyceride levels (7). There also appears to be a lower incidence of hypertension, and more hepatotropism with tacrolimus compared to cyclosporine (14).

There does not appear to be an increase in recurrence of hepatitis C virus (HCV); post-transplant lymphoproliferative disease appears no more frequent with tacrolimus therapy as compared to cyclosporine. There have been many reported cases of left ventricular cardiac hypertrophy associated with tacrolimus; it appears as though this hypertrophy may be reversible with either decreasing dose of tacrolimus or conversion to sirolimus.

Both tacrolimus and cyclosporine are associated with a high incidence of post-transplant diabetes mellitus (PTLD), reportedly occurring in up to 40% of patients. There does not appear to be any difference between tacrolimus and cyclosporine in terms of causing diabetes (7).

Occasionally adverse effects persist in spite of dose reduction; when this occurs a conversion from tacrolimus to cyclosporine often resolves these problems without increasing the incidence of acute or chronic rejection. In one study of 388 liver allograft recipients given tacrolimus, 70 patients required conversion to cyclosporine. The most common indications were neurotoxicity, diabetes mellitus, nephrotoxicity, gastrointestinal symptoms, cardiomyopathy and post-transplant lymphoproliferative disease. Improvement or resolution of these symptoms was seen in 90% of these patients after conversion, and there was no increased risk of rejection (15).

5.3. Sirolimus

Sirolimus, or rapamycin (Rapamune®, by Wyeth-Ayerst Laboratories) is a macrolide antibiotic first isolated from the species *Streptomyces hygroscopicus* from Rapa

Nui in the Easter Islands. It is similar in structure to tacrolimus and interacts with FKBP12 but appears to block T-cell activation at a point later in the pathway than tacrolimus by binding with the mammalian Target of Rapamycin (mTOR) protein. The major effect of this interaction is an inhibition of interleukin-2 (IL-2) – mediated signal transduction pathway, resulting in a suppression of T-cell activation and proliferation (16). Unlike cyclosporine and tacrolimus, sirolimus does not interact with calcineurin (16).

Other immunosuppressive effects include:

- Decreased T-cell response to IL-2, IL-12, IL-7 and IL-15.
- Decreased B-cell antibody production.
- Decreased thymocyte proliferation.
- Decreased IL-2R-based signal stimulation via p70^{56k} and p34.
- Inhibited progression of T-cells from Growth 1 (G₁) phase of the cell cycle to S phase.
- Decreased lipopolysaccharide-induced proliferation of B-cells via calcium-independent and FK-506/cyclosporine pathways.
- Decreased platelet-derived growth factor (PDGF) –stimulated proliferation of smooth muscle cells, which is thought to play a role in inhibiting chronic allograft rejection.

Adverse effects that result from sirolimus include hyperlipidemia (viz. hypercholesterolemia and hypertriglyceridemia), elevation in liver transaminases and bone marrow suppression (thrombocytopenia, leukopenia, anemia) (16).

One multi-center prospective randomized trial which compared cyclosporine and azathioprine versus sirolimus and azathioprine in renal allograft recipients found similar rates of acute rejection as well as comparable patient and graft survival at year post-transplant. The sirolimus treatment was associated with a statistically-significant higher incidence of hypertriglyceridemia, hypercholesterolemia, hypertransaminasemia, leukocytopenia, thrombocytopenia and arthralgia. However, the cyclosporine group had a higher mean serum creatinine, uric acid, phosphate and potassium and a lower calculated glomerular filtration rate (GFR). There was no statistical difference in rates of opportunistic infections (16). Thus sirolimus may provide similar efficacy as cyclosporine as maintenance therapy while avoiding the neuro- and nephrotoxicity associated with cyclosporine.

Sirolimus has some properties that make it unique among immunosuppressive drugs. First, it has been noted that sirolimus does not block activation-induced T-cell apoptosis as do cyclosporine and tacrolimus. This suggests that sirolimus may induce host tolerance, something seen in some earlier experimental transplant models used to investigate sirolimus. Second, sirolimus is purported to have anti-proliferative activity against neoplastic cells. This may possibly provide an advantage for recipients of liver allografts transplanted for hepatocellular carcinoma (17). Lastly, sirolimus may be useful to prevent and treat chronic

allograft rejection. Prevention of chronic rejection has been surmised from the fact that sirolimus decreases platelet-derived growth factor (PDGF)-stimulated proliferation of smooth muscle cells. Some effectiveness as a treatment for chronic rejection has been shown in one study (18), in which 50% of patients with biopsy-confirmed chronic rejection (eight of sixteen patients) showed resolution of their chronic rejection, determined by using levels of total bilirubin and transaminases as endpoints.

6. POLYCLONAL ANTIBODY THERAPY

The immunosuppressive effects of antilymphocyte serum have been known since the beginning of the 20th century. Subsequent observations showed that that polyclonal antirat lymphocyte serum prolonged skin graft survival in rats. Antilymphocyte sera (ALS) was first introduced into clinical use as an immunosuppressive by Starzl in 1967, and there is now more than 30 years of experience of use of polyclonal serum for use in transplant immunosuppression (14; 19). Since the development of monoclonal antibodies, the polyclonal antilymphocyte sera have not been used as frequently.

The first step in preparing polyclonal serum is obtaining lymphocytes or thymocytes – the latter often derived from either human cadavers or from the thymus glands removed during cardiac operations. The lymphocytes and thymocytes are screened for infectious diseases such as hepatitis B and C, human immunodeficiency virus (HIV), and human T-cell lymphocyte virus (HTLV) types I and II. Large animals (specifically, horses or rabbits) are immunized with purified suspensions of cultured human lymphocytes or thymocytes. Serum is then removed and fractionated to separate the globulin. Human red blood cells, obtained from Food and Drug Administration (FDA)-licensed blood banks, are used to deplete the serum from antibody that would react with non-T cell antigens. The serum is often pasteurized to achieve viral inactivation. The serum is then pooled to achieve consistency (19).

Equine antithymocyte globulin (ATGAM®, produced by Pharmacia & Upjohn, Inc.), is monomeric anti-human thymocyte IgG first introduced in 1972. The globulin produces lymphocyte depletion in a number of ways. Antilymphocyte antibodies binds to the surface of peripheral lymphocytes; complement-dependent opsonization and cytotoxic destruction then occurs in the spleen. Antibody-dependent cell-mediated cytotoxicity is thought to occur as well, possibly via Fas-mediated apoptosis. Those lymphocytes not lysed are suppressed via interaction of antibody with cell surface antigens. ATAGAM is given as an intravenous infusion once every other day for ten to fourteen days. The half-life of the serum ranges between two and nine days.

More recently, rabbit antithymocyte globulin (Thymoglobulin®, produced by SangStat), which had been used in Europe, was introduced into the United States. It has a similar mechanism of action and similar adverse effects; however, it has been shown to be more effective than ATGAM in reversing acute renal rejection.

ATGAM is typically infused via central venous catheter, vascular shunt or arteriovenous fistula, though several authors report infusing ATGAM through a peripheral vein without complications. It is recommended that a small (0.1 milliliter) intradermal injection of 1:1000 ATGAM be given alongside a saline control to assess for anaphylactic reactions (generalized rash, tachycardia, dyspnea or hypotension) which would preclude use of ATGAM.

The initial infusion is usually associated with a mild cytokine release syndrome consisting of chills, fever, myalgia and arthralgia. Patients are often premedicated with Solumedrol, acetaminophen and/or antihistamines. Other adverse effects included leukopenia, thrombocytopenia and anemia as a result of anti-blood cell component antibodies contained in the serum. An anaphylactic reaction to serum and "serum sickness" reactions occur as well.

Side effects of antibody therapy include the cytokine release syndrome, consisting of fevers, rigors, chills, and malaise, and, rarely, seizures, renal failure, pulmonary edema and cardiovascular death.

7. MONOCLONAL ANTIBODY THERAPY

7.1. OKT3

In 1986 orthoclone OKT3 (Muromonab CD3 by Ortho-Biotech) became the first monoclonal antibody approved by the Federal Drug Administration (FDA) for clinical use in transplantation. This drug is a murine monoclonal antibody specific for the ϵ -chain of the TCR/CD3 complex. In contrast to polyclonal antibody solutions it can be infused into a peripheral vein, making it very convenient. It is given once daily for ten to fourteen days. Monitoring is not necessary but may be done by checking serum Muromonab levels (therapeutic is $>1000\text{ng/mL}$) or white blood cell count (therapeutic $10\text{--}25\text{ cells/mm}^3$, whereas normal is $1000\text{--}1500$).

Orthoclone OKT3 causes a significant cytokine release (or "flu-like") syndrome after the first administration of the drug. The symptoms of this include fever, tachycardia, diarrhea, nausea, myalgia and dyspnea. It is caused by release of T-cell cytokines (TNF, IL-2, INF-gamma). There may be a slightly higher risk of hepatitis C recurrence and an increased risk of PTLD. Also, a large proportion of patients develop anti-horse antibodies, often as soon as by the end of the first course of treatment (i.e. after one week). These antibodies can neutralize the effects of OKT3 and therefore the immunosuppression the drug provides.

7.2. Daclizumab and Basiliximab

More recently, antibodies against the alpha chain of the IL-2a receptor (CD25, also known as Tac) have been developed. This receptor is expressed on the surface of nearly all activated T-cells but not on the surface of resting T-cells (20). Of the several subunits that together compose the IL-2R complex, including IL-2R-beta, IL-2R-gamma, it is only the IL-2R-alpha that is specific to IL-2R (21). With

this in mind, anti-Tac antibodies were developed in an attempt to cause inactivate or destruction of alloantigen-stimulated Tac-bearing lymphocytes (21).

The first anti-Tac monoclonal antibodies to be developed were murine in origin. The efficacy of these antibodies was limited by a short half-life and the nearly-uniform development of human anti-mouse antibodies (HAMAs). These problems were overcome with two newer monoclonal antibodies, daclizumab (Zenepax®, Hoffman-Roche) and basiliximab (Simulect®, Novartis). The former is human antibody, whereas the latter is a chimeric murine antibody (22). Basiliximab is less costly and requires only two doses given at post-transplant days 0 and 4. This results in saturation of IL-2R on circulating lymphocytes for 25–35 days. Daclizumab, on the other hand, requires five doses: one pre-operative dose and another four doses at two-week intervals. This dosing results in a saturation of the IL-2R on circulating lymphocytes for approximately 90 days (22). Neither of these two drugs has any reported side effects, and can safely be infused via a peripheral vein.

The effectiveness of both daclizumab and basiliximab has been studied with multi-center Phase III prospective randomized trials. Both drugs are associated with a decreased incidence of acute rejection and a decreased severity of acute rejection (as measured by need for antilymphocyte sera for treatment of acute rejection) during the first year after transplantation (22). When combined with cyclosporine, graft and patient survival exceeds 95% at six months (13).

8. PHARMACOLOGIC AGENTS IN DEVELOPMENT: FTY720

FTY720 (2-amino-2-(2-(4-octylphenyl)ethyl)-1,3-propanediol hydrochloride) is a less-toxic version of myriocin (ISP-1), an immunosuppressive compound initially isolated from culture filtrates of the ascomycete *Isaria sinclairii*. FTY720 results in both profound depletion of peripheral T- and B-lymphocytes and an inhibition of lymphocytes homing. It does not directly inhibit activation or proliferation of lymphocytes and does not directly inhibit cytokine production (23, 24).

The means by which FTY720 produces lymphocyte depletion is unknown. One hypothesis is that lymphocytes are effected to home toward lymph nodes and Peyer's patches, avoiding the allograft. Other ideas include interference with VLA-4 expression and with other cell-surface markers, especially chemokine receptors. It appears that FTY720 does not deplete the memory T-cell pool and will not lead to long-term allograft tolerance (23, 25).

FTY720 is a sphingosine analogue. It is presumed that it disrupts the sphingolipid pathway, a pathway that has been associated with lymphocyte signal transduction and differentiation and in apoptosis.

FTY720 has a long absorption period, not reaching C_{max} until 24 hours after administration. Peripheral blood lymphocytes are reduced in number

within six hours of administration of the drug. Numbers are reduced by approximately 30% within 72 hours of administration (25). The drug is metabolized by the liver in animal models; metabolism in humans has not yet been well studied (23).

There are four adverse effects that have been associated with FTY720: (1) orthopnea; (2) non-productive dry cough; (3) herpes zoster; (4) increase in liver enzymes. There has also been an associated bradycardia especially when given concurrently with beta-blockers, possibly resulting from an effect on receptors of the sinus node. There is no known renal, pancreatic or bone marrow toxicity associated with the drug (23).

9. SUMMARY

Immunosuppression regimens have advanced since the inception of organ transplantation. The first effective regimen of azathioprine and steroids allowed for the first successes in kidney and liver transplantation. Polyclonal sera allowed for the better induction and treatment of acute rejection. The adverse effects of this early regimen, however, were significant: patients were maintained on high doses of steroids, and the profound immunosuppression led to many opportunistic infections.

The next major advance came with the introduction of cyclosporine in 1983. Graft survival improved, and incidence of acute rejection during the first post-transplant year decreased. Continued improvement in graft survival followed with the use development of tacrolimus, and alternative to cyclosporine, and mycophenolate, and alternative to azathioprine. Most recently, research into monoclonal antibodies and chimeric molecules have led to the development of basiliximab and daclizumab as treatment for acute rejection or induction with virtually no adverse effects.

The current immunosuppression medications used for maintenance therapy, however, are not yet ideal – that is, they do not achieve the goal of inducing graft tolerance while avoiding generalized immunosuppression and non-immunologic adverse effects. Future research will likely focus on molecular- and gene-level mechanisms to achieve this goal.

10. REFERENCES

1. Pirsch JD & ED Neto: Mycophenolate Mofetil and Azathioprine. In: Current and Future Immunosuppressive Therapies Following Transplantation. Eds: Sayegh MH, G Remuzzi. Kluwer, Dordrecht, Netherlands (2001)
2. Rayhill SC & HW Sollinger: Mycophenolate Mofetil: Experimental and Clinical Experience. In: Immunosuppression in Transplantation. Eds: Ginns LC, AB Cosimi, & PJ Morris, Blackwell Science, Malden, MA (1999)
3. Stuart FP: Immunosuppression. In: Organ Transplantation. Eds: Stuart FP, MM Abdecassis & DB Kaufman, Landes Bioscience, Georgetown, TX (2000)
4. Marino IR & HR Doyle: Conventional immunosuppressive drugs. In: Immunosuppressive Drugs: Developments in anti-rejection therapy. Eds: Thomson AW & TE Starzl, Edward Arnold, London (1994)
5. Goldfien A: Adrenocorticosteroids and Adrenocortical Antagonists. In: Basic and Clinic Pharmacology. Ed: Kantzung BG, Appleton & Lange, Stanford, CT (1998)
6. McPhee SJ: Disorders of the Adrenal Cortex. In: Pathophysiology of Disease. Eds: McPhee SJ, VR Lingappa, WF Ganong & JD Lange. Appleton & Lange, Stanford, CT (1997)
7. Fernandez LA, R Lehmann, L Luzi, A Battessati., MC Angelico, C Ricordi, A Tzakis & R Alejandro: The Effects of Maintenance Doses of FK506 Versus Cyclosporin A on Glucose and Lipid Metabolism After Orthotopic Liver Transplantation. *Transplantation* 68, 1532-1541 (1999)
8. Almond PS: Kidney Transplantation. In: Organ Transplantation. Eds: Stuart FP, MM Abdecassis & DB Kaufman, Landes Bioscience, Georgetown, TX (2000)
9. Franklin JE: Psychiatric Issues in Organ Transplantation. In: Organ Transplantation. Eds: Stuart FP, MM Abdecassis & DB Kaufman, Landes Bioscience, Georgetown, TX (2000)
10. Cronin DC, TW Faust, L Brady, H Conjeevaram, S Jain, P Gupta & JM Millis: Modern Immunosuppression. *Clin Liver Dis* 3, 619-655 (2000)
11. Stark T: Knife to the Heart: The Story of Transplant Surgery. Macmillan, London (1996)
12. Hong JC & BD Kahan: The History of Immunosuppression for Organ Transplantation. In: Current and Future Immunosuppressive Therapies Following Transplantation. Eds: Sayegh MH, G Remuzzi, Kluwer, Dordrecht, Netherlands, (2001)
13. Keown PA: Molecular and Clinical Therapeutics of Cyclosporine in Transplantation. In: Immunosuppression in Transplantation. Eds: Ginns LC, AB Cosimi & PJ Morris, Blackwell Science, Malden, MA (1999)
14. Klintmalm GB: Induction and Maintenance of Immunosuppression. In: Transplantation of the Liver. Eds: Busuttil RW & GB Klintmalm. W.B. Saunders Co., Philadelphia, PA (1997)
15. Emre S, Genyk Y, Schluger LK, TM Fishbein, SR Guy, PA Sheimer, ME Schwartz & CM Miller: Treatment of tacrolimus-related adverse effects by conversion to cyclosporine in liver transplant recipients. *Transpl Int* 13, 73-78 (2000)
16. Groth CG, L Backman, JM Morales, R Calne, H Kreis, P Lang, JL Touraine, K Claesson, JM Campistol, D Durand, L Wramner, C Brattstrom & B Charpentier: Sirolimus (Rapamycin)-Based Therapy in Human Renal Transplantation. *Transplantation* 67, 1036-1042 (1999)

17. Watson CJE, PJ Friend, NV Jamieson, TW Frick, G Alexander, AE Gimson & R Calne: Sirolimus: A Potent New Immunosuppressant for Liver Transplantation. *Transplantation* 67, 505-509 (1999)
18. Nishida S, A Pinna, R Verzaro, D Levi, T Kato, F Khan, J Nery, D Weppeler & A Tzakis: Sirolimus (Rapamycin)-Based Rescue Treatment Following Chronic Rejection After Liver Transplantation. *Transplant Proc* 33, 1495 (2001)
19. Morrissey P & AP Monaco: Polyclonal Antilymphocyte Therapy. In: Current and Future Immunosuppressive Therapies Following Transplantation. Eds: Sayegh MH, G Remuzzi. Kluwer, Dordrecht, Netherlands (2001)
20. Vincenti F, R Kirkman, S Light, G Bumgardner, M Pescovits, P Halloran, J Neylan, A Wilkinson, H Ekberg, R Gaston, L Backman & J Burdick: Interleukin-2-Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation. *N Engl J Med* 338, 161-165 (1998)
21. Vincenti F, M Lantz, J Birnbaum, M Garovoy, D Mould, J Hakimi, K Nieforth & S Light: A Phase I Trial of Humanized Anti-Interleukin 2 Receptor Antibody in Renal Transplantation. *Transplantation* 63, 33-38 (1997)
22. Vincenti FG: Monoclonal Antibody Targeting of IL-2R-Complex. In: Current and Future Immunosuppressive Therapies Following Transplantation. Eds: Sayegh MH, G Remuzzi. Kluwer, Dordrecht, Netherlands (2001)
23. Brinkmann V, R Schmouder, L Feng & D Pinschewer: FTY720: A Novel Class of Immunosuppressant. *Graft* 2, 187-188 (1999)
24. Napoli KL: The FTY720 Story. *Ther Drug Monit* 22, 47-51 (2000)
25. Kahan BD: Update on Pharmacokinetic/Pharmacodynamic Studies with FTY720 and Sirolimus. *Therapeutic Drug Monitoring* 24, 47-52 (2002)

Key Words: Immunosuppression, Calcineurin Inhibitors, Monoclonal Antibody Therapy, Polyclonal Antibody Therapy, Corticosteroids, Review

Send correspondence to: John A. Goss, M.D., Michael E. DeBakey Department of Surgery, Baylor College of Medicine, 6550 Fannin, Suite 1628, Houston TX 77030, Tel: 713-798-8355, Fax: 713-798-7762, E-mail: jgoss@bcm.tmc.edu