J Am Soc Nephrol 10: 1366–1380, 1999

# Newer Immunosuppressive Drugs: A Review

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*Abstract.* In recent years, many new immunosuppressive drugs have been discovered and developed for clinical use in transplantation. This review focuses on those drugs (leflunomide, mycophenolate mofetil, sirolimus, tacrolimus) that have been shown to have immunosuppressive activity in patients. Different anti-interleukin-2 receptor antibodies are also reviewed as an example of a resurgence of development in the area of monoclonal antibodies. The price for reducing the incidence of allograft rejection by improved immunosuppression was thought to be a proportional increase in the incidence of infection and malignancy. Data from Phase III clinical trials of new immunosuppressants, however, show a statistically significant reduction in the incidence of acute rejection produced by these new drugs, which has not been accompanied by increases in infection and malignancy rates. The wide array of new drugs offers the opportunity to use combinations that block different pathways of immune activation while at the same time selecting drug combinations with nonoverlapping toxicity profiles so that doses of each single drug can be reduced below toxicity levels. The immunosuppressive therapy for patients can be tailored according to their individual needs.

In the 1990s, many new small and large molecules have been discovered and developed for use as immunosuppressants in solid organ transplantation. This review focuses on those drugs that have proven immunosuppressive activity in patients (1,2). Tacrolimus (FK 506) and mycophenolate mofetil (MMF) have already replaced immunosuppressive maintenance protocols at some institutions. The other two drugs, leflunomide and sirolimus (SRL), are still under investigation for use in solid organ transplantation. Anti-interleukin-2 (IL-2) receptor antibodies have shown promising results in phase III trials. Conventional wisdom has held that the price for reducing the incidence of allograft rejection by improved immunosuppressants is a proportional increase in the incidence of infection and malignancy. When the data from Phase III trials of new immunosuppressants are analyzed, however, the statistically significant reduction in the incidence of acute rejection produced by these new drugs has not been accompanied by proportional increases in infection and malignancy rates in the first year after transplantation. Because most of the new immunosuppressants reviewed in this chapter differ in their mechanisms of action, and because the toxicities are mechanism-based, the wide array of new drugs offers the opportunity to use combinations that block different pathways of immune activation while at the same time selecting combinations with nonoverlapping toxicity profiles so that doses of each drug can be reduced below toxic levels. The development of so many novel and very different small molecule and monoclonal antibody immunosuppressants will enable the transplant physician to tailor therapy for individual patients more precisely than ever before. Designing individualized regimens, however, presumes that the clinician understands the many facets of this new world of immunosuppression. This review has been prepared to provide a foundation for this understanding.

## Leflunomide and Malononitriloamides

### *Background*

Leflunomide and the malononitriloamides (MNA) are a new class of immunomodulating drugs that are currently under investigation for use in transplantation. In 1985, the anti-inflammatory and immunomodulating properties of leflunomide were recognized, which differ from classical anti-inflammatory and immunosuppressive drugs. The immunosuppressive effects of leflunomide have been investigated extensively in animal models of transplantation. Because of its long half-life (11 to 16 d) in humans, the clinical development of leflunomide has been restricted to use in patients with autoimmune diseases such as rheumatoid arthritis. A large preclinical program has been started to evaluate the potential use of the leflunomide analogues HMR 715 and HMR 279. These analogues, malononitriloamides, are very similar in structure to the active metabolite of leflunomide, A77 1726, and may have a more favorable pharmacokinetic profile.

### *Pharmacokinetics*

Leflunomide [*N*-(4-trifluoro-methylphenyl)-5-methylisoxazol-4-carboxamide] is a synthetic isoxazole derivative that is metabolized in the gut and liver to its main metabolite, the malononitriloamide A77 1726. This pharmacologically active metabolite is stable and represents more than 90% of the metabolites in the serum in animals and humans. It is hydrophilic and readily soluble in water. There is still little information about the pharmacology of leflunomide, and no data exists

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1046-6673/1006-1366
Journal of the American Society of Nephrology

regarding MNA in humans. The bioavailability of leflunomide in rabbits is close to 100% after oral administration. The plasma to whole blood ratio is one. A77 1726 is primarily associated (95%) with the lipoprotein-free fraction of plasma (3). In rats, the peak drug level is reached after 8 to 12 h. In humans, leflunomide has a half-life between 5 and 18 d (4), and the plasma clearance rate is 0.3 ml/kg per h. HPLC methods are available to measure plasma concentrations of A77 1726 and the other MNA.

### *Pharmacodynamics*

The effects of A77 1726 and the other MNA appear to be identical (5). Leflunomide suppresses T cell and B cell proliferation *in vitro* (6) and inhibits the proliferation of smooth muscle cells *in vitro* (7,8). The primary known effect of the MNA is the inhibition of protein tyrosine kinases and DHODH, a critical enzyme for the *de novo* pyrimidine synthesis. Activated lymphocytes need both the *de novo* pathway and the salvage pathway to synthesize a sufficient amount of pyrimidines to proliferate.

Protein tyrosine kinases play a critical role at various steps in the signal transduction pathways, including mitogenesis and transformation (9). However, much higher concentrations of A 77 1726 are needed to block the tyrosine kinase activity than to inhibit lymphocyte proliferation *in vitro*. In vitro, DHODH is inhibited by A 77 1726 in the nanomolar or low micromolar range (10). At concentrations that block cell proliferation, A77 1726 inhibits DHODH; the antiproliferative effects can be antagonized by pyrimidine nucleotides (6).

The antiproliferative potency of A77 1726 is species- and cell type-dependent. Rat lymphocytes are the most sensitive and human lymphocytes are the least sensitive. More direct evidence for A77 1726 interfering with the *de novo* pyrimidine biosynthetic pathway *in vivo* comes from murine studies. Treatment of mice with leflunomide, but not cyclosporin A (CsA), reduces DHODH activity in lymphocytes infiltrating heart allografts (11). Although the administration of 20 mg/kg leflunomide prolonged nonvascularized heart to ear transplants in mice, the coadministration of leflunomide with high doses of uridine resulted in mean survival times similar to untreated control animals.

*In vitro* and *in vivo* experiments in allotransplantation and xenotransplantation showed that A77 1726 prevents antibody production (8,12–14). The effects of A77 1726 on cytokine synthesis or growth factor receptor expression are contradictory and are dependent on the cell line, the type of mitogen, and the A77 1726 concentration. Most studies have shown that antiproliferative concentrations of A77 1726 have a minimal effect on cytokine production and cytokine receptor expression (15–17).

### *Animal Studies*

Leflunomide has been investigated extensively in numerous animal models of transplantation and autoimmune diseases, such as tubulointerstitial nephritis in rats (18). The prevention of acute allograft rejection has been tested in mice (heart), rats (heart, skin, intestine, lung, myocutaneous), dogs (kidney), and monkeys (heart).

When administered for 7 d in the heterotopic rat heart model (Brown Norway to Lewis), leflunomide prolonged graft survival with doses as low as 0.63 mg/kg. Administration of 5 mg/kg over 21 d resulted in a 50% rate of indefinite graft survival (19).

Prolonged graft survival (36 d) was achieved in a study in cynomolgus monkeys with heterotopic heart transplants (8), when leflunomide was given in a daily dose of 15 mg/kg. Ongoing acute rejection in heterotopic heart transplants between different rat strains was successfully treated with leflunomide doses between 5 and 20 mg/kg (20).

In rat models for prevention and treatment of chronic rejection, leflunomide inhibited graft vascular disease in heart, aorta, and femoral vessel allografts. The delayed treatment with leflunomide halted the progression of preexisting graft vascular disease (5,20–23).

Leflunomide has been tested in several models for concordant and discordant xenotransplantation. In the hamster to rat heart transplant model, graft survival up to 76 d was achieved with a dose of 15 mg/kg (24). In the guinea pig to rat heterotopic heart transplantation model, leflunomide in combination with cobra venom factor resulted in the longest graft survival (129 h) reported in this model (25).

### *Clinical Trials*

Available data from human trials with leflunomide are entirely from Phase I and II trials in rheumatoid arthritis. Oral doses between 10 and 25 mg/patient per d were effective compared with placebo. A total of 402 patients was enrolled in the Phase II prospective randomized trial to access the safety and effectiveness of leflunomide. A dose-dependent improvement in the primary and secondary outcome measures was observed (4). For the MNA, clinical data are not available.

### *Adverse Effects and Toxicity*

The most important side effect in cynomolgus monkeys was anemia (8). In the Phase II leflunomide study, adverse effects included gastrointestinal symptoms, rash and allergic reactions, weight loss, and reversible alopecia. The incidence of infections in the leflunomide group was not increased; decreases in hematocrit and hemoglobin were observed in all groups.

## Mycophenolate Mofetil

### *Background*

Mycophenolic acid (MPA) was initially derived from cultures of Penicillium spp. by Gosio (26) in 1896 and purified by Alsberg and Black in 1913. Antibacterial and antifungal activities were recognized in the 1940s. Antitumor activity was described in 1968 (27), and MPA was further studied for psoriasis (28), but did not gain clinical use. Mitsui and Suzuki (29) demonstrated its potential immunosuppressive properties, but the failure to prolong mouse skin graft survival substantially delayed its further study as an immunosuppressant. The rapid metabolism of MPA in mice in contrast to other species (*e.g.*, rats) accounted for its early experimental failure. These

species differences in half-lives led to its reevaluation in rats as an immunosuppressant for allograft recipients and prompted the first studies to show its efficacy for this indication (30–33).

Further developmental work produced an ester prodrug of MPA, mycophenolate mofetil (MMF), which demonstrated a higher bioavailability in cynomolgus monkey than MPA (34). Early clinical studies in cadaveric kidney (35) and liver transplantation (36) showed promising results. In 1995, MMF was approved by the U.S. Food and Drug Administration for prevention of acute renal allograft rejection. In 1998, approval was granted for its use in heart transplant recipients. Despite the variety of other novel purine (mizoribine) and pyrimidine (leflunomide and MNA, brequinar) inhibitors recently developed for transplantation, MMF is currently the leading candidate for replacement of azathioprine.

### *Pharmacokinetics*

MMF, the 2-morpholinoethyl ester of MPA, is a prodrug. It is rapidly and completely hydrolyzed into its active metabolite MPA after oral administration by plasma esterases. The parent compound is not measurable in plasma *[*z](34). MMF shows free solubility in alcohol, but is only slightly soluble in water. The volume of distribution of MPA in healthy volunteers is 3.6 L/kg (37) after oral or intravenous administration. The ratio of the oral and intravenous area under the curve is 94% (37). At clinically relevant concentrations, MPA is almost completely (>99%) bound to plasma albumin (38). Therefore, plasma is the matrix of choice for measurement of MPA concentrations (39). MPA is metabolized to mycophenolic acid glucuronide (MPAG) by uridine diphosphate-glucuronosyl transferase in the liver, and MPAG is the primary urinary excretion product of the drug. Approximately 87% of the drug is eliminated in urine; 6% is eliminated in the faeces (37,39). MPAG is only a weak inosine monophosphate dehydrogenase (IMPDH) inhibitor. The MPAG inhibitory concentrations ($IC_{50}$) with recombinant IMPDH were found to be 532- to 1022-fold higher than those for MPA (40). However, in another study MPAG $IC_{50}$ values for inhibition of human lymphocyte IMPDH were only 10-fold higher compared with MPA (41). Other unidentified metabolites are suspected to be pharmacologically active (40,42).

MPA undergoes substantial enterohepatic circulation, contributing to its gastrointestinal toxicity. MPAG is converted by mucosal enzymes and gut flora to MPA and is reabsorbed. This results in secondary peaks in pharmacokinetic studies after 6 to 12 and 24 h (39). For clinical use, MPA plasma concentrations are measured by enzyme multiplication immunoassay technique. The necessity of therapeutic drug monitoring is still under investigation (43).

### *Pharmacodynamics*

MPA is a highly selective noncompetitive and reversible inhibitor of IMPDH. IMPDH is a crucial enzyme in the *de novo* biosynthesis of guanosine. Inhibition of IMPDH causes a depletion of guanine nucleotides (44). Proliferating lymphocytes differ from most other cells in that they are fully dependent on both the *de novo* pathway and the salvage pathway of purine biosynthesis. Most other cell lines can maintain their function with the salvage pathway alone. Due to this property of lymphocytes and the high specificity of MPA for IMPDH compared with other nicotinamide adenine dinucleotides (45), MPA is a very specific lymphocyte inhibitor.

MPA inhibits proliferation of both T and B lymphocytes (46) in response to mitogenic and allospecific stimulation. The inhibitory effect can be reversed *in vitro* (peripheral human blood lymphocytes and lymphoma cell lines) by adding guanosine or desoxyguanosine (44). Antibody formation in humans to horse antilymphocyte globulin is also inhibited by MMF (47). In human spleen cells stimulated by tetanus toxoid, antibody formation is inhibited even after adding MPA at day 3 (46,48).

Guanosine nucleotides are necessary for glycosylation of lymphocyte and monocyte glycoproteins; by inhibiting guanosine nucleotide synthesis, glycosylation of adhesion molecules is suppressed. The inhibition of migration to sites of rejection or inflammation may be impaired. *In vitro* studies in human cell lines have shown that MPA inhibits the incorporation of mannose and fucose into cellular glycoproteins (49). Human monocytes exposed to MPA demonstrate decreased adherence to endothelial cells or extracellular protein matrix (50).

### *Animal Studies*

The first promising animal study of MMF was in the heterotopic heart transplantation rat model. A dose of 40 mg/kg per d administered over 50 d posttransplant resulted in indefinite survival of the graft, and 20 mg/kg per d resulted in a 50-d survival (30,33). In the same model, the combination of CsA (0.75 mg/kg per d) and MMF (10 mg/kg per d) produced at least an additive effect with a graft survival over 50 d. Either drug alone resulted in a graft survival of only 10 to 11 d (32,33). In a cynomolgus monkey heart allograft model with MMF doses between 70 and 175 mg/kg per d, prolongation of graft survival could be achieved (19 to 62 d compared with 9 d in controls) (32). Ongoing rejection could also be reversed when MMF was given at the time of rejection (33).

MMF has been found to decrease graft vascular disease in a chronic heterotopic heart rat model (32). In renal (51) and aortic (52) transplantation models, chronic rejection was reduced. Furthermore, MMF was effective in reducing antibody-mediated rejection in the rat heterotopic heart model (53).

In animal models of concordant cardiac xenotransplantation, MMF showed only a very limited improvement in graft survival (54), and in discordant xenotransplant MMF had no beneficial effect (55).

### *Clinical Trials*

The first clinical studies were done in 1992 (safety and efficacy Phase I trials) and showed that oral doses of MMF from 100 to 3500 mg/d were well tolerated. There was a significant correlation between rejection episodes and low MPA blood levels (56). In 1995, results were published from the first placebo-controlled study of this agent. In Europe, MMF was combined with CsA and steroids for prevention of

acute rejection in cadaveric renal transplantation. A total of 491 patients was enrolled in this multicenter trial with three treatment arms (placebo, MMF 2 g/d, and MMF 3 g/d). This study showed that MMF significantly reduced the rate of biopsy-proven rejection or other treatment failure during the first 6 mo after transplantation. Overall, the frequency of adverse events was similar in all treatment groups. Gastrointestinal problems, leukopenia, and opportunistic infections were more common in the MMF groups, and there was a trend toward more events with higher doses (57). The results from a U.S. study with 499 renal transplant patients were comparable. Biopsy-proven acute rejection episodes or treatment failure occurred in 47.6% of patients in the azathioprine group compared with 31.1% in the 2-g MMF and 3-g MMF groups (35). The tricontinental (Australia, Europe, United States) study in cadaver kidney transplant recipients showed that MMF significantly reduced the incidence of rejection episodes in the first 6 mo after transplantation. Significant improvement in graft survival could not be demonstrated (58). A pooled analysis of all three studies showed a significant decrease in acute rejection episodes (40.8% [placebo/azathioprine] *versus* 16.9% [2 g MMF] and 16.5% [3 g MMF]), but no significant improvement in 1-yr graft survival (90.4% [2 g MMF] and 89.2% [3 g MMF] compared with 87.6% [placebo/azathioprine]) (59). MMF substituted for azathioprine has been shown to be effective in treating recurrent or persistent cardiac allograft rejection (60,61).

In the most recent multicenter heart trial, 28 centers enrolled 650 patients. MMF (3 g/d) was tested *versus* azathioprine (1.5 to 3 mg/kg per d). Comparing treated patients, in the MMF group the 1-yr mortality was 6.2% *versus* 11.4% in the azathioprine group, and the requirement for rejection treatment was significantly reduced (65.7% *versus* 73.7%). However, opportunistic infections were more common in the MMF group (62).

Currently, MMF is used in patients who have contraindications for azathioprine (such as the need for allopurinol) or as the primary choice of an antimetabolite. Based on the experience in clinical trials, the recommended initial dose is 2 g/d divided in two doses.

In a preliminary retrospective case-control study in kidney allograft recipients with established chronic rejection, adding MMF to maintenance immunosuppression provided no clear benefit (63).

### *Adverse Effects and Drug Toxicity*

The primary toxic side effects are anemia in rats and leukopenia, diarrhea, and anorexia in dogs and monkeys, and these side effects can be reduced by lowering the dose. The most common side effects of MMF in humans are diarrhea, vomiting, opportunistic infections, and leukopenia. The mechanism of myelotoxicity is not well understood. Because of selective inhibition of the *de novo* pathway of purine synthesis, MPA should affect only proliferating lymphocytes. In contrast to transplant recipients, patients treated with MMF for psoriasis rarely develop leukopenia (64).

## Sirolimus

### *Background*

Sirolimus (rapamycin, SRL), a microbial product isolated from the actinomycete *Streptomyces hygroscopicus*, was discovered initially as an antifungal agent in the mid-1970s (65). Because of its immunosuppressive effects, it was not further developed for clinical use as an antibiotic. The advent of tacrolimus and the recognition of the structural similarities between these two drugs led two research groups independently to study its immunosuppressive properties in experimental organ transplantation (Figure 1) (66,67).

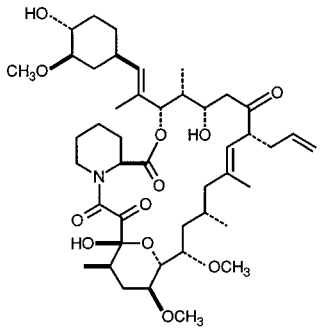
### *Pharmacokinetics*

Structurally resembling tacrolimus, SRL contains the same tricarbonyl region including an amide, a ketone, and a hemiketal, but a triene segment in SRL differentiates these two drugs. Because of this structural difference, SRL is a hydrophobic drug that has low stability in aqueous solutions. A new SRL derivative, SDZ-RAD, has been developed with about two to three times lower *in vitro* potency, but *in vivo* potency not different from that of SRL (68). When administered orally to human kidney recipients, SRL was absorbed rapidly with a peak blood concentration at 1.4 h (69). Oral bioavailability of SRL is 15% in kidney transplant recipients, and the mean half-life is about 60 h (70,71). In the blood, more than 95% of the drug is bound to red blood cells (72). The drug is widely distributed into tissue stores (73). SRL is metabolized by the cytochrome P450 system and more than 10 metabolites have been identified, some of them with low immunosuppressive activity *in vitro* (74–76). HPLC methods can detect SRL concentrations in the ng/ml range, and newly developed HPLC/electrospray-mass spectroscopy methods detect as low as 0.25 ng/L (77).

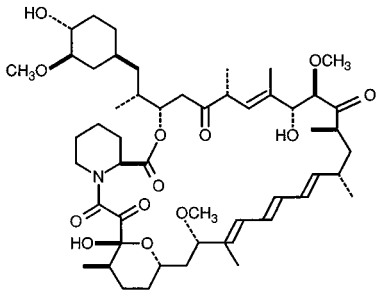
### *Pharmacodynamics*

Because it is lipophilic, SRL passes through cell membranes easily, and the segment of the macrolactam ring identical to tacrolimus binds to cytosolic FK506-binding proteins (FKBP). The consequent mechanisms of action for tacrolimus-FKBP12 and SRL-FKBP complexes differ in several ways (78,79). Unlike tacrolimus, SRL does not inhibit calcineurin phosphatase, but its molecular targets include RAFT1/FRAP proteins in mammalian cells, associated with cell cycle progression through $G_1$; however, the exact mechanism of inhibition of cell cycle progression through these proteins is still unknown (Figure 2) (80–83).

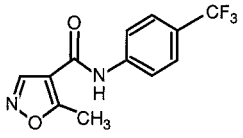
Another possibly even more effective way to prolong the cell cycle at the $G_1$/S interface is the ability of SRL to selectively inhibit the synthesis of ribosomal proteins and to inhibit the induction of mRNA for new ribosomal proteins. These effects are mediated by inactivation of p70 s6 kinase ($p70^{s6k}$), specifically the sites of action associated with phosphorylation (79,84–87). In addition, SRL inhibits IL-2-induced binding of transcription factors in the proliferating cell nuclear antigen promoter, thus inhibiting cell cycle progression. As a conse-



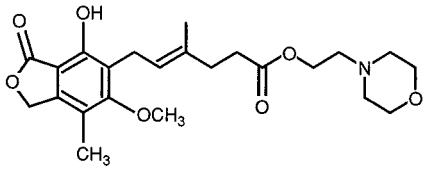

**TACROLIMUS**
**(FK 506)**




**SIROLIMUS**
**(RAPAMYCIN)**




**LEFLUNOMIDE**




**MYCOPHENOLATE MOFETIL**

*Figure 1.* Molecular structures of different immunosuppressive xenobiotics. Modified from reference (1).

quence of its inability to interfere with early events after T cell activation, SRL is a less effective inhibitor of cytokine synthesis than CsA and tacrolimus (88,89).

On the other hand, SRL inhibits several of the CsA-resistant pathways in both T and B cell stimulation (90). SRL inhibits B cell Ig synthesis and antibody-dependent cellular cytotoxicity, as well as lymphocyte-activated killer cells and natural killer cells (91,92).

A characteristic feature of SRL is its ability to inhibit growth factor signaling for both immune and nonimmune cells (93–95). This antiproliferative effect includes at least fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells. This antiproliferating effect of SRL renders it (at least theoretically) a promising compound for the prevention of chronic rejection (93,94,96). Slight interaction between prednisolone and SRL has been observed in stable human kidney transplant recipients, and potent interaction has been observed between SRL and CsA during *in vivo* animal studies (97,98). SRL and CsA show synergism in immunosuppression both *in vitro* and *in vivo* (98–103).

### *Animal Studies*

Efficacy of SRL has been proven in several animal models, many of them in large animals. It prolonged kidney allograft survival in dogs, and in pigs was at least as effective as cyclosporin-based immunosuppression (65,104–108). As a monotherapy, it prolonged graft survival in different cardiac allograft models (109–112). Transplant vasculopathy is significantly inhibited in a heterotopic rat cardiac transplant model and in transplanted femoral artery allografts in a dose-dependent manner (95,113,114). SRL has proven effective in large animal kidney allograft models, but reports of toxicity have been more frequent compared with rodent models (105–108). In cynomolgus monkeys, abdominal heart allograft survival is prolonged by SRL monotherapy (65,115). SRL effectively reverses ongoing allograft rejection in several solid organs including the kidney (116). SRL can also induce strain-specific long-term tolerance in the rat (117–119). In xenografting, SRL alone has only a limited effect on prevention of hyperacute or acute xenograft rejection, but it appears to potentiate the effect of other drugs when used in combination (120–122).

### *Clinical Studies*

Clinical studies with SRL immunosuppression have mainly been published from kidney transplant recipients (70,71,123). Phase I studies suggest interindividual variations in the pharmacokinetic parameters in stable renal transplant patients, indicating that optimal use in humans may require monitoring of drug concentrations (124). SRL has been reported in the use of rescue therapy for refractory renal allograft rejection in human kidney recipients (123).

### *Adverse Effects and Toxicity*

The current profile of adverse effects in humans is mainly predicted based on preclinical studies and Phase I and II studies in stable kidney recipients (70,125). Headache, nausea, dizziness, changes in blood glucose level, epistaxis, infection, and decrease in platelets and white blood cells have been described in association with short-term SRL administration (70,110,126,127). One concern may be hypertriglyceridemia, which has been reported in association with long-term (several months) use of rapamycin (128).

The nephrotoxicity associated with tacrolimus and CsA was avoided by SRL in several studies in rats and in pigs possibly

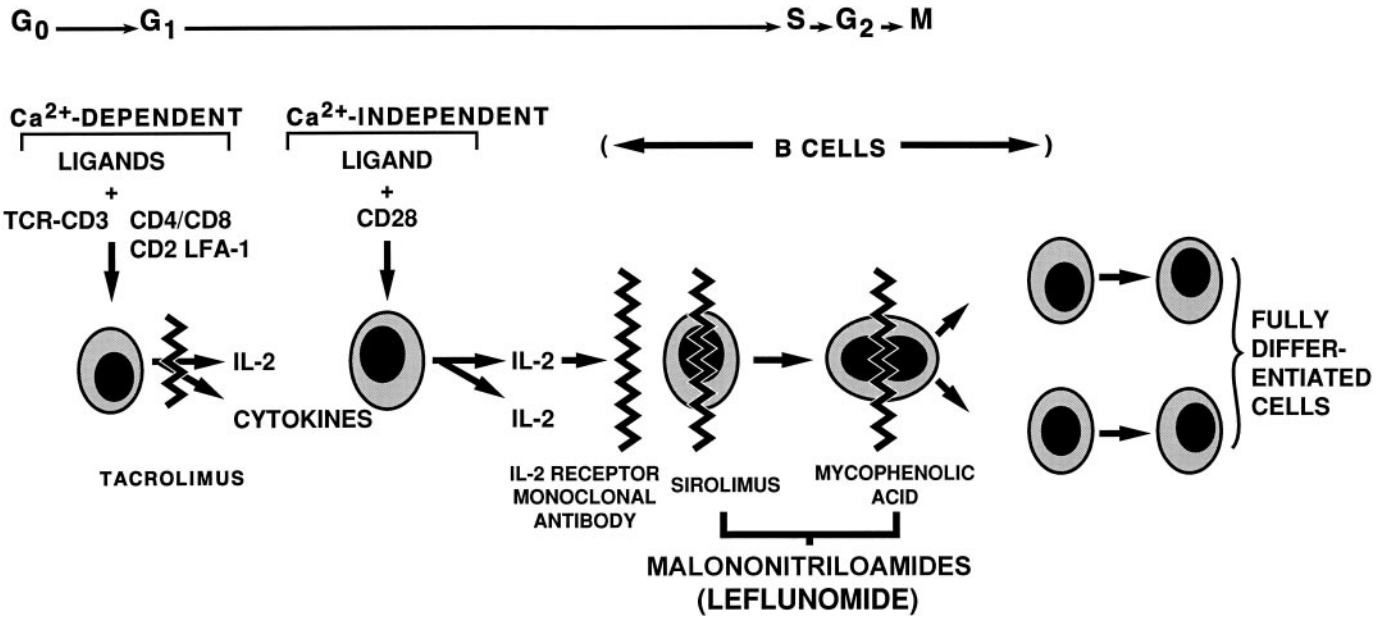



*Figure 2.* Possible sites of action in lymphocytes of new immunosuppressants. TCR, T cell receptor. Modified from reference (1).

due to the lack of calcineurin inhibition (129–132). However, hypomagnesemia and tubular injury were side effects in normal rats receiving SRL, and progression of kidney failure in spontaneously hypertensive rats has been described (133). Myocardial and retinal infarctions have been described in rats after a high dosage of SRL (132,134). In dogs, severe gastrointestinal toxicity with mucosal necrosis and submucosal vasculitis has been described (105,135). Severe vasculitis was also seen in primates (106).

## Tacrolimus (FK506)

### *Background*

Tacrolimus, a metabolite of an actinomycete *Streptomyces tsukubaensis*, was first demonstrated to be immunologically effective *in vivo* in rat heart allograft recipients in 1987 (136,137). It was soon found to be a potent alternative to CsA in several experimental models.

### *Pharmacokinetics*

Because tacrolimus is minimally soluble in aqueous solvents, it is formulated in alcohol and a surfactant for continuous intravenous administration (138). The oral formulation is composed of capsules of a solid dispersion of tacrolimus in hydroxypropyl methylcellulose (139). Absorption of tacrolimus is incomplete after oral administration. Its bioavailability ranges from 10 to 60%, with peak blood levels after 1 to 2 h and half-life of 8 to 24 h (140–142). The oral dose of tacrolimus needs to be higher than intravenous doses. Administration of tacrolimus by the intravenous route leads to a rapid distribution of the drug reflected as a rapid decline of the initial peak concentration, followed by a slower decline over the next 24 h (143). Tacrolimus is highly bound to plasma proteins, *e.g.*, albumin, and to red blood cells and lymphocytes (144,145). Most of the solid organs exhibit a high concentration of tacrolimus, particularly the lungs, heart, kidney, pancreas, spleen, and liver. The major part of the metabolism takes place in the intestinal wall and in the liver by the cytochrome P450 system (146,147). At least 15 metabolites have been detected, and some of them show pharmacologic activity (148,149). Drug level monitoring is required, because tacrolimus has high inter- and intraindividual variability and a narrow therapeutic index (142). Drug levels can be monitored by an enzyme-linked immunosorbent assay or by RIA from whole blood (150,151).

### *Pharmacodynamics*

The mechanism of action is similar for tacrolimus and CsA (98,152–154). The process is initiated by binding of the tacrolimus molecule to cytoplasmic immunophilins, FKPB, of which the isoform FKBP12 seems to be involved in the immunosuppressive effect caused by tacrolimus (155–157). The tacrolimus-FKBP complex inhibits the activity of calcineurin, a serine-threonine phosphatase that regulates IL-2 promoter induction after T cell activation (158,159). Inhibition of calcineurin impedes calcium-dependent signal transduction, and inactivates transcription factors (NF-AT) that promote cytokine gene activation, because they are direct or indirect substrates of calcineurin's serine-threonine phosphatase activity (160,161). As a consequence, the transcription of cytokines IL-2, IL-3, IL-4, IL-5, interferon-$\gamma$, tumor necrosis factor-$\alpha$, and granulocyte-macrophage colony-stimulating factor, and IL-2 and IL-7 receptors, is suppressed by tacrolimus (162–165).

Tacrolimus inhibits lymphocyte activation *in vitro* 10 to 100 times more potently than CsA (165). One explanation might be the higher binding affinity of tacrolimus to FKPB compared to the binding of CsA to its immunophilin called cyclophilin (156). Other immunosuppressive effects of tacrolimus include

the inhibition of T cell proliferation and the inhibition of primary or secondary cytotoxic cell proliferation *in vitro*, whereas direct cytotoxicity and calcium-independent T cell stimulation are not affected (166,167). Tacrolimus also suppresses B cell activation *in vitro*: both induced Ig production by B cells and the proliferation of stimulated B cells (168). *In vivo*, tacrolimus inhibits proliferative and cytotoxic responses to alloantigens and suppresses primary antibody responses to T cell-dependent antigens, whereas secondary antibody responses, IL-2-stimulated cell proliferation, and natural killer or antibody-dependent cytotoxic cell function are not inhibited (169–171).

### *Animal Studies*

Tacrolimus was first described as a promising immunosuppressive agent to control acute rejection in experimental heart transplantation in rats (172). Later studies showed its efficacy for suppression of heart allograft rejection in nonhuman primates (113,173–175). Controversial results have been published concerning the role of tacrolimus in prevention of chronic rejection. In a heterotopic rat cardiac transplant model, high dose tacrolimus treatment reduced the incidence of cardiac allograft vascular disease (176), whereas other studies showed that tacrolimus was not able to prevent graft-vessel disease (113,177). In a rat hind limb transplant model, tacrolimus was superior to SRL or CsA in prolongation of allograft survival (178).

Tacrolimus has been shown to prolong the survival of concordant heart xenografts in a hamster to rat model when combined with antiproliferative drugs or splenectomy (54,179,180), as well as in a concordant model in primates (181).

### *Clinical Trials*

Tacrolimus has been investigated in clinical transplantation of all solid organs, and it has been approved as an immunosuppressant agent for primary therapy in patients with liver and kidney transplants. In renal transplantation, tacrolimus was used first in 1989 in Pittsburgh (182). Many clinical trials and reports in renal allograft recipients have been published (183–189). Tacrolimus has been proven effective in patients with steroid-resistant rejection episodes. In the most recent randomized, comparative multicenter trial including 412 patients, tacrolimus was equivalent to CsA in 1-yr graft and patient survival. The number and severity of biopsy-proven acute rejection episodes were significantly lower in the tacrolimus group (190). After 3 yr, patient and graft survival was still equivalent for both groups, but the number of graft failures defined as loss of graft excluding death was significantly lower in the tacrolimus group. A higher overall incidence of post-transplant diabetes mellitus was observed in the tacrolimus group (191).

### *Adverse Effects and Toxicity*

Significant nephro- and neurotoxicity have been reported in patients receiving tacrolimus treatment (192–194). One possible mechanism for the neurotoxicity is the inhibition of calcineurin phosphatase, but the etiology of its renal vasculopathic effects is unclear. Reduced renal glomerular and cortical blood flow and increased renal vascular resistance are generally associated with increased thromboxane A2, endothelin production, or stimulated intrarenal renin production (192). Cardiomyopathy, anemia, chronic diarrhea, onset of diabetes, and allergies have been reported in patients receiving tacrolimus (195,195). Compared with CsA, hypercholesteremia and hypertension are less common, and gingival hyperplasia and hirsutism are notably absent in patients receiving chronic tacrolimus treatment (192,194,195). Lymphoproliferative disease and infections are associated with tacrolimus-based immunosuppressive protocols (195,196).

## IL-2 Receptor Monoclonal Antibodies

### *Background*

In the late 1960s, the introduction of polyclonal T cell antibodies (antilymphocyte globulin, antithymocyte serum, antithymocyte globulin) was a breakthrough in solid organ transplantation leading to prolonged graft survival. Because of the nonspecific immunosuppression achieved with polyclonal antibodies and the increased knowledge about rejection and T cell activation, research was directed at the development of specific monoclonal T cell antibodies.

The first commercially available monoclonal antibody was OKT3 in 1981 (mouse CD3). It is used routinely for both induction therapy and rejection therapy. Because OKT3 is a nonhuman protein and because of its interaction with all lymphocytes, there are significant side effects in patients treated with OKT3, including cytokine release syndrome and malignancies (197). Recent studies have been focusing on more specific monoclonal antibodies, thereby reducing the side effects (198–200). Another major achievement is the development of chimeric and humanized monoclonal antibodies, thus reducing the immunogenicity and increasing human immune effector functions (201). The important role of the IL-2/IL-2 receptor system in lymphocyte proliferation and the selective expression of this receptor on activated T lymphocytes led to investigation of the IL-2 receptor as a target for monoclonal antibody therapy.

### *Pharmacodynamics*

The high-affinity IL-2 receptor consists of three noncovalently bound chains: a 55-kD $\alpha$-chain (CD 25, Tac), a 75-kD $\beta$-chain, and a 64-kD $\gamma$-chain (202). The $\alpha$-chain is expressed only on activated T lymphocytes. The clonal proliferation of activated T cells is suppressed by blocking CD25. Hypothetically, by binding the antibody with CD25 the receptor cannot be activated by free IL-2. The expression of the IL-2 receptor may be downregulated.

The weak performance of specific murine monoclonal antibodies is caused by a rapid development of neutralizing antibodies against the monoclonal antibodies in about 80% of the recipients (200). In addition, the ability of murine antibodies to interact with the human complement system to lyse cells can be impaired. So-called humanized or chimeric antibodies could overcome this limitation. They do not elicit an antibody reac-

tion and are able to interact with the human complement system.

### *Animal Studies*

Kirkman *et al.* demonstrated in 1987 a prolongation of murine cardiac allograft survival by the anti-IL-2 receptor monoclonal antibody AMT-13 (203). Prolongation of kidney allograft survival in cynomolgus monkeys has been achieved with use of an anti-Tac monoclonal antibody (204).

### *Clinical Trials*

A variety of IL-2 receptor antibody studies have been performed in humans with kidney or heart transplantation. A rat IgG2a monoclonal antibody, 33B3.1, prevented renal allograft rejection as effectively as antithymocyte globulin, but with better tolerance (199).

Anti-Tac, a murine IgG2a monoclonal antibody directed against the $\alpha$-chain of human IL-2 receptors, combined with standard CsA therapy showed a marked reduction in the incidence of early renal graft rejection. However, no improvement in either graft or patient survival could be demonstrated (205,206). BT 563, a murine IgG1 anti-IL-2 receptor antibody, has also been shown to effectively prevent rejection after kidney transplantation without infectious complications or side effects (207). BT 563 has also been used in an open-label randomized study in heart transplant recipients with disappointing results attributed to the late onset of CsA therapy and to the redundancy of the cytokine network (208,209).

A new generation of humanized IL-2 receptor antibodies has recently been introduced. Daclizumab (HAT [humanized anti-Tac] or Zenapax®) is a genetically engineered humanized IgG that binds to the $\alpha$-chain of the IL-2 receptor. Results from Phase I and III trials in kidney transplants are encouraging. Daclizumab significantly reduced the incidence of acute rejection in kidney transplant patients (210).

Another antibody used for prophylaxis in a Phase III clinical trial of cadaver kidney transplant patients (211) is basiliximab (Simulect®), a chimeric (human and mouse) monoclonal antibody directed against the $\alpha$-chain of the IL-2 receptor. It is produced *in vitro* by continuous culture fermentation of a murine-myeloma cell line transfected with plasmid-borne recombinant gene construct coding for murine variable regions and human constant regions. Basiliximab—given on day 1 and day 4 (20 mg)—was tested against placebo. There was a significantly lower rejection rate in the basiliximab group, and the steroid dosage could be reduced.

### *Adverse Effects and Drug Toxicity*

IL-2 receptor antibodies were well tolerated and have almost no side effects compared with OKT3. No evidence of cytokine release syndrome was seen. The infection rate was comparable to the placebo group, and no significant difference regarding malignancies was observed in these short-term studies.

## Summary

More effective and specific immunosuppressive therapy is needed to further reduce the high morbidity due to infections, malignancies, and graft loss due to chronic rejection after kidney transplantation. Two different approaches to improve immunosuppression are under way: the development of new small molecules as immunosuppressants and the development of targeted monoclonal antibodies. Another strategy is the monitoring of immunosuppressive therapy by pharmacodynamic markers. The ultimate goal of immunosuppressive therapy—its elimination through the development of allograft-specific tolerance—has not been reproducibly achieved and may never be realized for all patients. Perhaps the immune systems of most patients will be able to be regulated by a more sophisticated combination of several immunosuppressive drugs, antibodies, and donor cells to become specifically hyporesponsive. By reducing the need of nonspecific immunosuppressants, the frequency of infections, malignancies, and drug toxicity can be diminished, and a clinically acceptable and more realistic alternative to complete "tolerance" may become available.

## Acknowledgments

Dr. Gummert is supported by Deutsche Forschungsgemeinschaft Grant Gu 472/1-1. Dr. Ikonen is supported by The Jalmari and Rauha Ahokas Foundation and the Academy of Finland. Drs. Gummert, Ikonen, and Morris are supported by Hedco Foundation and the Ralph and Marian Falk Trust.

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