

Transplantation

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Tacrolimus

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HISTORY

Tacrolimus (FK506, Prograf; Fujisawa, Deerfield, IL, USA) is a macrolide compound isolated from *Streptomyces tsukubaensis*, a soil fungus that can be found in Northern Japan (1). Its immunosuppressive properties were first recognized in 1984 during a screening program directed at identifying new immunosuppressive agents. Subsequent *in vitro* and animal experiments in Japan and at the University of Pittsburgh helped to characterize the mechanism of action of tacrolimus and demonstrated its potent immunosuppressive properties in transplantation (1–6).

IMMUNOLOGY

Most clinically useful immunosuppressive drugs either inhibit the proliferation of T lymphocytes or destroy them. Cyclosporine inhibits cytokine synthesis by binding to cytoplasmic proteins (cyclophilins) in T lymphocytes (see Chapter 6). Tacrolimus functions in a similar fashion, binding to FK-binding proteins (FKBP) in the cytoplasm; however, tacrolimus has a greater binding affinity to FKBP than does cyclosporine to cyclophilin. Rapamycin, which is structurally similar to tacrolimus, does not inhibit cytokine synthesis but rather inhibits the response of T cells to interleukin 2 (IL-2) and other cytokines (see Chapters 6 and 11).

The complex formed by the binding of tacrolimus to FKBP-12 associates with calcium-dependent calcineurin/calmodulin complexes to impede calcium-dependent signal transduction subsequent to stimulation of calcium influx in lymphocytes. Transcription factors that promote cytokine gene activation are direct or indirect substrates for calcineurin, and their activity is reduced by association with the tacrolimus complex. Tacrolimus inhibits the mixed lym-

phocyte reaction assay, the formation of IL-2 by T lymphocytes, and formation of other soluble mediators including IL-3, IL-4, IL-5, tumor necrosis factor alpha (TNF- α), and granulocyte-macrophage-colony stimulating factor (7). Tacrolimus also inhibits the expression of IL-2 and IL-7 receptors. As an immunosuppressive agent, tacrolimus is approximately 100 times more potent than cyclosporine (8).

PHARMACOKINETICS AND MONITORING

Tacrolimus achieves adequate absorption from the upper small intestine following oral administration, but the extent of absorption is widely variable (range = 5% to 67%) among patients. Administration with food generally does not affect the oral absorption of tacrolimus, although fatty meals may reduce bioavailability (9). Because absorption is bile independent, intravenous administration is not required in most patients (as is often required with cyclosporine [Sandimmune], particularly during the early postoperative period), and dosage changes are not required when clamping a T-tube following liver transplantation (10).

During liver graft failure or hepatic dysfunction, especially with cholestasis, tacrolimus bioavailability increases and clearance decreases owing to its extensive hepatic metabolism. The result is markedly elevated tacrolimus levels with related toxicity necessitating a rapid reduction in dosage (10,11). In contrast, initial dosing reductions usually are not required in patients with renal impairment (10).

Tacrolimus concentrations can be measured either in whole blood or plasma using an enzyme-linked immunosorbent assay (ELISA) (12) or in whole blood with a micro-particle enzyme immunoassay (13). Measurement of plasma tacrolimus concentrations may be less desirable both because of the complexity of the methodology and because plasma must be separated at 37°C due to temperature-dependent

distribution of tacrolimus into erythrocytes, similar to that of cyclosporine (14). Importantly, plasma levels of tacrolimus measured by ELISA were found to correlate poorly with rejection and toxicity (15), whereas whole blood tacrolimus levels were well correlated with rejection and toxicity (16). Other studies indicated a higher correlation of plasma tacrolimus levels with clinical events (17). As with cyclosporine, plasma level monitoring can be used if samples are handled carefully. However, because of the technical difficulties inherent in plasma level monitoring, whole blood levels are usually considered the standard of care.

During oral therapy with tacrolimus, concentrations should be monitored to maintain 12-hour trough levels in the range of 5 to 15 ng/mL for whole blood or 0.5 to 1.5 ng/mL for plasma to optimize efficacy and minimize toxicity. Clinical experience has shown that high trough tacrolimus levels correlate with a lower incidence of rejection, and high peak levels correlate with increased toxicity

(18,19). A definite correlation between tacrolimus levels and nephrotoxicity has not been recognized, but a reduction in serum creatinine levels was reported in liver transplant recipients when tacrolimus levels were reduced (17).

PRECLINICAL EXPERIENCE WITH TACROLIMUS

Numerous animal studies have been conducted demonstrating the *in vitro* and *in vivo* effects of tacrolimus (Table 9.1) (20). Although many studies have evaluated the use of tacrolimus in preventing graft rejection, the published literature has also described the effects of tacrolimus on the inhibition of cell-mediated responses, proliferative responses, cytotoxic responses, and humoral responses, as well as its effect on various autoimmune disorders.

An *in vitro* study evaluated the effect of tacrolimus compared to cyclosporine and prednisolone on the mixed lym-

Table 9.1. Experimental Autoimmune Diseases Suppressed by Tacrolimus

Disease	Species	Tacrolimus Dose (mg/kg/day unless specified)	Reference
Arthritis (Type II collagen-induced)	Rat (Lewis)	0.32 ^{ab}	Inamura et al, 1988 (21)
	Rat (Outbred)	2.5 ^c	Arita et al, 1990 (22)
	Mouse (DBA/1)	2.0	Takagishi et al, 1989 (23)
Type 1 diabetes	NOD mouse	2.0 mg/kg/48 h ^a	Miyagawa et al, 1990 (24)
	Cyclophosphamide-treated NOD mouse	0.2, 1.0, 2.0	Carroll et al, 1991 (25)
	BB rat	1.0 ^a	Murase et al, 1990 (26)
	BB rat	25 µg IM ^d	Nicoletti et al, 1991 (27)
Uveoretinitis	Rat (Lewis)	1.0 ^{ae}	Kawashima et al, 1990 (28)
	Rhesus and cynomolgus monkeys	0.5 ^f	Fujino et al, 1990 (29)
Thyroiditis	Rat (PVG)	2.0 ^g	Tamura et al, 1992 (30)
Lupus (SLE)	MRL-lpr/lpr mouse	2 mg ^h	Yamamoto et al, 1988 (31)
	NZB/NZW F ¹ mouse	2.5 mg/kg/48 h ⁱ	Takabayashi et al, 1989 (32)
Glomerulonephritis	Rat (Wistar)	0.3 mg ^j	Hara et al, 1990 (33)
(Nephrotoxic antiserum nephritis)	Rat (Wistar)	0.64	Okuba et al, 1990 (34)
Heymann nephritis	Rat (Wistar)	0.64	Okuba et al, 1990 (34)
	Rat (Lewis)	1.0	Matsukawa et al, 1992 (35)
Allergic encephalomyelitis	Rat (Lewis)	1.0	Inamura et al, 1988 (36)
Autoimmune myocarditis	Rat (Lewis)	1.0, 0.32, 1.0	Hanawa et al, 1992 (37)
Experimental allergic contact dermatitis	Farm pig	0.04, 0.4% topical	Meingassner and Stutz, 1992 (38)
Murine (coxsackie B ³) myocarditis	Mice (C3H/He)	2.5	Hiraoka et al, 1992 (39)

^a Suppresses induction of disease.

^b Partially effective during efferent phase of response.

^c On day of immunization.

^d Administered daily from 27–120 days of age.

^e Effective only in induction phase.

^f Administered from 3 weeks after immunization.

^g Administered for 3 weeks following induction of disease.

^h Administered from 8 weeks of age.

ⁱ From time of immunization.

^j Administered 5 days per week after immunization.

^k Administered from day 0–13 or 56–69.

Adapted from Thomson AW, Murase N, Nalesnik MA, Starzl TE. The influence of tacrolimus on experimental autoimmune disease. In: Lieberman R, Mukherjee A, eds. Principles of drug development in transplantation and autoimmunity. Austin, TX: RG Landes, 1996.

phocyte reaction (MLR) (2). Tacrolimus demonstrated dose-dependent suppression of the proliferative response of human lymphocytes to alloantigen stimulation at concentrations higher than 0.1 nmol/L. The IC_{50} values of tacrolimus, cyclosporine, and prednisolone were 0.22, 14.0, and 80.0 nmol/L respectively. Tacrolimus was also shown to inhibit IL-2 production almost completely at concentrations higher than 0.3 nmol/L. With an IC_{50} value of approximately 0.1 nmol/L, tacrolimus was reported to be approximately 100 times more potent than cyclosporine, which had an IC_{50} value of 10 nmol/L. Tacrolimus demonstrated a similar inhibitory effect on interferon (IFN)- γ production.

In a separate study, tacrolimus inhibited the proliferation of murine spleen B lymphocytes induced by anti-mouse IgM by approximately 50% *in vitro* (40).

In vivo animal studies have investigated the effect of tacrolimus on humoral and cellular immunity. In one study, mice were immunized with sheep erythrocytes and received tacrolimus and cyclosporine orally for 4 days from the day of immunization (1). After 4 days, tacrolimus almost completely suppressed splenic antibody-forming cell (AFC) response at doses of 10 mg/kg or more, whereas cyclosporine almost completely suppressed AFC response at 100 mg/kg. Tacrolimus also suppressed delayed-type hypersensitivity (DTH) responses dose-dependently in mice immunized with methylated bovine serum albumin (MBSA).

The effect of tacrolimus on transplantation of the liver, kidney, heart, islet cells, and abdominal visceral organs has been widely studied in different animal models (41–46). In a liver transplantation study using beagle dogs, 8 of 10 dogs (80%) receiving tacrolimus survived longer than 30 days, providing an early *in vivo* demonstration that tacrolimus, 1.0 mg/kg, was as potent as cyclosporine, 20 mg/kg (42). Tacrolimus exhibited similar immunosuppressive effects in cynomolgus monkeys undergoing orthotopic liver transplantation (43).

Tacrolimus has also shown promise in animal models of cardiac transplantation. The hearts of F344 rats were heterotopically transplanted into the cervical region of WKA rat recipients receiving either tacrolimus or cyclosporine orally (4). Both agents prolonged acceptance of the cardiac allografts. Tacrolimus significantly prolonged graft survival in rats receiving a heterotopic cardiac transplantation across a strong major histocompatibility complex (MHC) barrier at a dose of 1.28 mg/kg/day (44). When a moderate histoincompatible combination was used, the animal recipients survived indefinitely.

In renal transplantation studies using tacrolimus in unrelated beagle dogs, all the animals in the control group died of renal failure due to graft rejection within 24 days; whereas, animals treated with 1.0 mg/kg/day oral dosage of tacrolimus survived over 140 days after transplantation (3). The effect of tacrolimus in kidney transplantation was also studied in baboons (46). In the tacrolimus groups, 3 of 5 (60%) and 4 of 5 (80%) animals survived longer than 80

days, depending on the dose. All of the animals in the control group died within 14 days of renal failure due to graft rejection. This preclinical trial in subhuman primates suggested that tacrolimus was a promising drug for use in humans.

Preclinical studies of tacrolimus in intestinal transplantation also have been promising (47,48). The efficacy of tacrolimus in several experimental animal models of allotransplantation is summarized in Table 9.2 (49).

EARLY CLINICAL EXPERIENCE IN TRANSPLANTATION

Most early clinical experience with tacrolimus was gained from the pioneering work at the University of Pittsburgh in liver, renal, heart, and small bowel transplantation. Initially, tacrolimus was used as rescue therapy in patients experiencing rejection or toxicity while being treated with cyclosporine. Subsequent trials were expanded to the use of tacrolimus as primary immunosuppression (52–54). This early experience helped to identify the safety and tolerability of tacrolimus as well as its role as primary and rescue therapy following transplantation (Table 9.3).

CLINICAL EXPERIENCE WITH TACROLIMUS FOR SOLID ORGAN TRANSPLANTATION

Liver

Until recently, most clinical experience with tacrolimus had come from its use in liver transplantation as either primary or rescue therapy. Two prospective randomized trials conducted in the United States and Europe found that patient and graft survival were comparable with tacrolimus and cyclosporine but that the incidence of rejection, both acute and steroid refractory, was significantly lower with tacrolimus (55,56).

The US open-label, randomized, multicenter trial compared the efficacy and safety of tacrolimus to a cyclosporine (Sandimmune)-based immunosuppressant regimen in patients undergoing primary liver transplantation (55). Adult and pediatric patients were randomized at the time of transplant to tacrolimus ($n = 263$) or cyclosporine ($n = 266$) and followed for 12 months. Study end points were patient and graft survival and the incidence of acute, steroid-resistant, and refractory rejection. Patient and graft survival at 1 year were comparable for the tacrolimus and the cyclosporine groups (Fig. 9.1). Acute and steroid-resistant rejection and treatment failure due to refractory rejection were significantly lower with tacrolimus. Longer-term follow-up of these recipients now suggests a survival benefit for the tacrolimus-treated patients, compared to the Sandimmune-treated group (57). There also appears to be a significant benefit for the tacrolimus-treated liver allograft recipients with hepatitis C. Their 5-year survival rate was 78%, versus only 60% for the Sandimmune-treated HCV-positive patients.

Table 9.2. Tacrolimus in Experimental Allotransplantation

Animal	Organ (Reference)	Strain		Dose (mg/kg/day)	Tacrolimus Route	Duration (Days)	Mean Survival (Days)			
		Donor	Recipient							
Rat	Heart (50)	ACI	LEW	0.0	—	—	6.0			
				0.6	IM	0–13	39.0			
				1.3	IM	0–13	87.0			
	Liver (41)	ACI	LEW	1.3	IM	4,5,6	91.0			
				0.0	—	—	10.0			
				1.3	IM	4,5,6	>100.0			
	Intestine (47)	BN	LEW	0.0	—	—	11.7			
				0.6	IM	0–13	>100.0			
				1.3	IM	0–13	>100.0			
		Multivisceral (51)	BN	LEW	0.0	—	—	12.0		
					0.6	IM	0–13	27.3		
					1.3	IM	0–13	>100.0		
Dog	Kidney (5)	Mongrel	Beagle	0.0	—	—	13.0			
				0.5	PO	1–90	33.7			
				1.0	PO	1–90	31.0			
				1.5	PO	1–90	61.0			
				2.0	PO	1–90	32.1			
				1.0	IM	4,5,6	58.0			
	Liver (5)	Beagle	Beagle	0.0	—	—	12.4			
				1.0	PO	1–90	66.5			
				1.0	IM	4,5,6	96.6			
				Intestine (42)	Mongrel	Mongrel	0.0	—	—	7.8
							0.1	IV	1–90	57.5
							0.2	IV	1–90	23.3
Baboon	Kidney (46)	<i>Papio abunus</i>	<i>Papio abunus</i>	1.0	IM	3,4,5	17.7			
				0.0	—	—	9.2			
				6.0	PO	1–90	29.5			
				12.0	PO	1–90	70.8			
				18.0	PO	1–90	74.6			
2.0	IM	4,5,6	53.3							

LEW = Lewis; BN = Brown-Norway.

Adapted from Todo S and Murase N. Tacrolimus for experimental organ transplantation. In: Lieberman R, Mukherjee A, eds. Principles of drug development in transplantation and autoimmunity. New York: RG Landes, 1996.

Table 9.3. Potential Uses for Tacrolimus

Transplantation
Liver
Kidney
Heart
Intestine
Bone marrow
Lung
Autoimmune Diseases
Psoriasis
Uveitis
Chronic inflammatory diseases of the liver
Nephrotic syndrome

Among pediatric patients, there was a trend toward less rejection and a reduced requirement for steroids with tacrolimus, although patient and graft survival were comparable (58). Major neurologic events and diarrhea were more common with tacrolimus, while hirsutism and higher serum cholesterol levels were reported with cyclosporine.

In a parallel study conducted at eight European transplant centers, 545 patients were randomized to tacrolimus or a conventional cyclosporine (Sandimmune)-based regimen (56). The primary end points were acute, refractory acute, and chronic rejection at 12 months. The rates of rejection were significantly lower with tacrolimus. Patient and graft survival rates were not different between tacrolimus and cyclosporine. The mean corticosteroid dosage

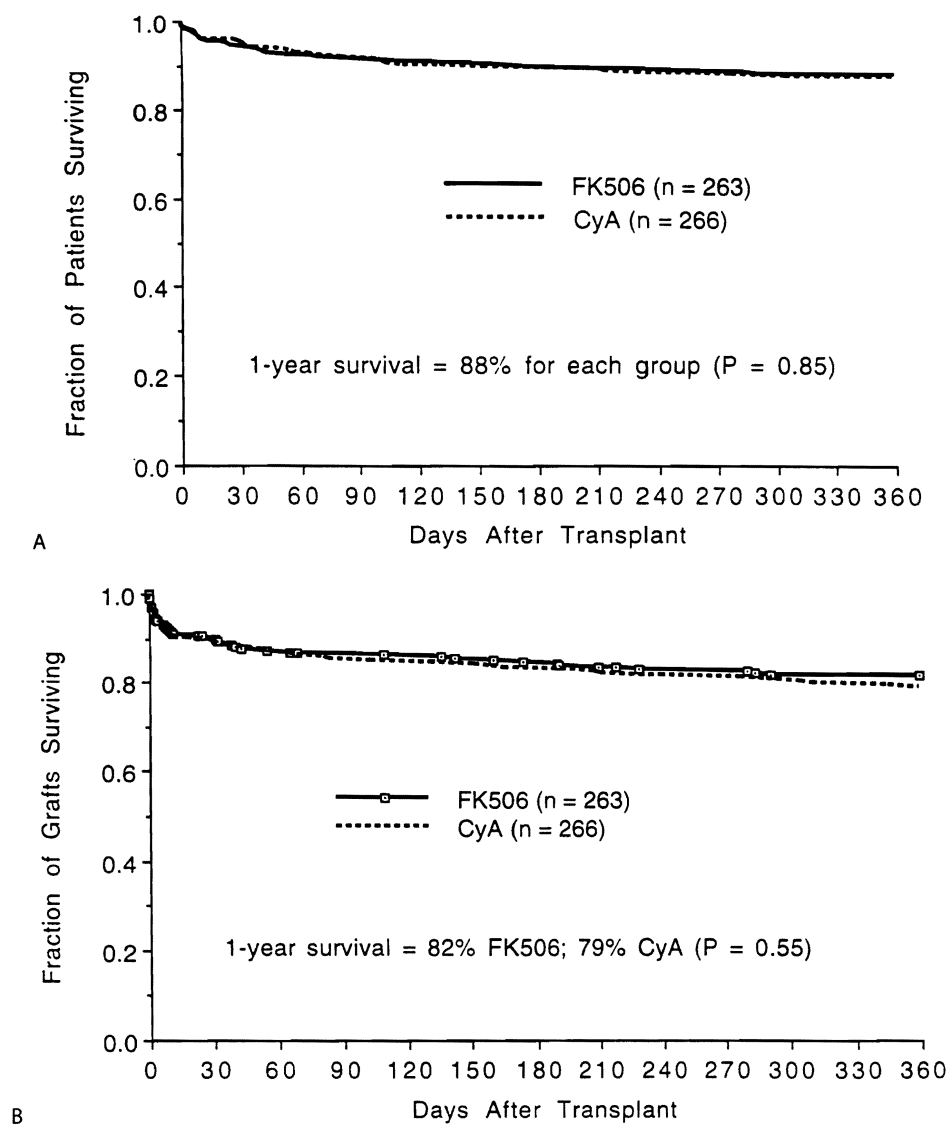


FIGURE 9.1. Patient survival (A) and graft survival (B) in patients treated with FK506 or cyclosporine-based (CsA) immunosuppression (55). Kaplan-Meier estimates of 1-year patient and graft survival after liver transplantation were not significantly different between treatment groups.

was significantly lower and almost no azathioprine or anti-lymphocyte globulin was required in the tacrolimus group compared with the cyclosporine group.

In the European experience, patients transplanted for fulminant hepatic failure had a particularly impressive response to tacrolimus (59). At 1 week, patient survival was 95.5% with tacrolimus and 82.1% with cyclosporine; by 6 months, survival was 72.7% with tacrolimus and 60.7% with cyclosporine (Fig. 9.2). Similar results were observed for graft survival. Also, the incidence of treated rejection episodes was significantly reduced with tacrolimus. This resulted in a lower mean daily corticosteroid requirement for tacrolimus- versus cyclosporine-treated patients (69 mg/day versus 150 mg/day) and was reflected in a lower incidence of infectious complications with tacrolimus.

A limitation of these studies was the comparison of tacrolimus to Sandimmune, rather than to Neoral therapy (see also Chapter 6). More recent trials suggest a lower incidence of rejection in tacrolimus-treated liver allograft recipients in comparison to those treated with Neoral, but these studies have not yet been completed.

Rescue Therapy Following Liver Transplantation

Tacrolimus in combination with corticosteroids proved to be an effective immunosuppressant regimen for rescue therapy in patients with rejection on a cyclosporine-based regimen after liver transplantation (53). Included were 72 patients with acute rejection, 131 with chronic rejection, and 43 patients with either co-morbid disease or severe cyclosporine-related side effects. At a median follow-up of

240 days, 87% of patients were alive and 79% had functioning grafts. Graft loss was greatest among patients with chronic rejection and a serum bilirubin >2.5 mg/dL (28%) at the start of tacrolimus therapy and among those with viral hepatitis (19%).

An open-label, multicenter study evaluated the safety and efficacy of tacrolimus plus corticosteroids for rescue therapy in 300 adult and 86 pediatric liver transplant recipients experiencing refractory rejection despite conventional immunosuppression (60). All patients were followed for 1 year after conversion to tacrolimus. Of these patients, 260 completed a 1-year follow-up on tacrolimus. Patient survival at 1 year was 74.5% for adults and 79.8% for children, and

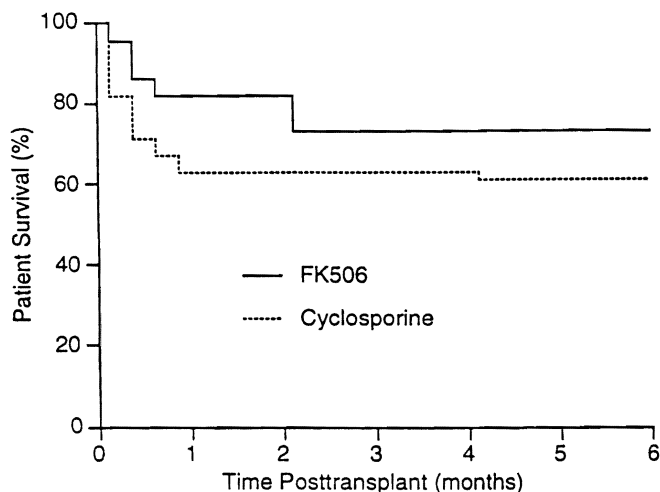


FIGURE 9.2. Patient survival following liver transplantation for fulminant hepatic failure after treatment with FK506 or cyclosporine (59).

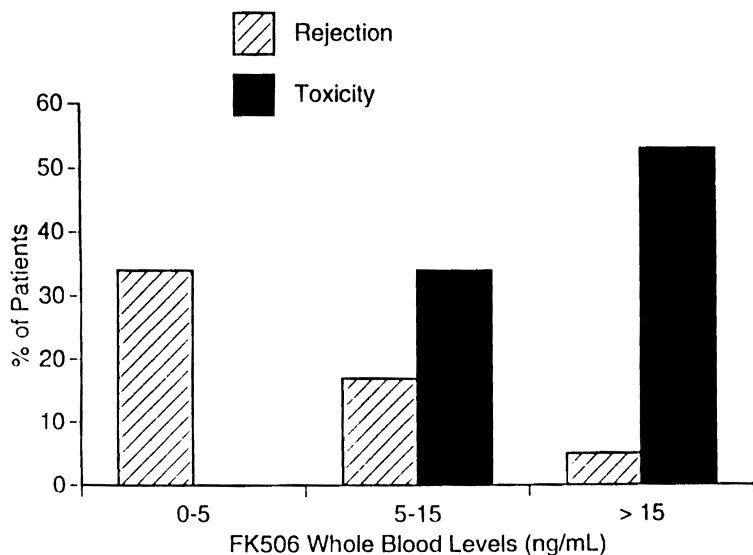


FIGURE 9.3. Incidence of toxicity and rejection by whole blood FK506 levels (19). A logistic regression analysis was performed to determine the relationship between the occurrence of rejection and toxicity, and the whole blood concentrations of FK506. The occurrence of toxicity requiring a dosage reduction increased significantly with increasing levels of whole blood FK506. Conversely, the likelihood of the occurrence of rejection decreased significantly with increasing whole blood levels of FK506. These data support a recommended target whole blood FK506 level of 5 to 15 ng/mL during the first 42 days of treatment.

graft survival at 1 year was 64.7% for adults and 67.9% for children. When outcomes were stratified by baseline total bilirubin levels >6 or <6 mg/dL and by acute versus chronic rejection, patient and graft survival were consistently lower in patients with bilirubin levels >6 mg/dL.

Kidney

An early multicenter, open-label trial compared various tacrolimus dosages with cyclosporine in 120 patients undergoing primary cadaveric kidney transplant (19). Patients were randomized to a cyclosporine-based regimen or to one of three tacrolimus-based regimens designed to achieve low (5 to 14 ng/mL), medium (15 to 25 ng/mL), or high (26 to 40 ng/mL) trough whole blood levels. This trial revealed that the incidence of neurotoxic and gastrointestinal events was higher among tacrolimus-treated patients during the first month after transplant and correlated with increasing maximum trough tacrolimus concentrations ($P = 0.01$). Also noted was a decreasing rate of rejection with increasing minimum trough tacrolimus concentrations at 1 month ($P = 0.021$) (Fig. 9.3). The range of tacrolimus whole blood levels that optimized efficacy and minimized toxicity was found to be 5 to 15 ng/mL corresponding to an initial oral dose of 0.2 mg/kg/day. At 1 year, patient survival was 98% for all tacrolimus-treated patients and 92% for the cyclosporine group. Graft survival was 93% and 89% in the tacrolimus and cyclosporine groups, respectively (61). The incidence of rejection episodes requiring treatment during the first year was 33% for tacrolimus and 32% for cyclosporine. Until day 42, the incidence of acute rejection was significantly lower (14% tacrolimus, 32% cyclosporine; $P = 0.048$) for the aggregate of all tacrolimus patients versus cyclosporine. In the tacrolimus group, 13 rejection episodes occurred within the first 6 weeks and 17 more were reported at 1 year. In the cyclosporine group, no new rejec-

tion episodes were reported beyond 42 days after transplantation. Nephrotoxicity occurred with a similar frequency with tacrolimus and cyclosporine, but the incidence of neurotoxic events and the incidence of new insulin use were higher among tacrolimus-treated patients.

A subsequent randomized, open-label US study compared the efficacy and safety of optimal dosage tacrolimus to cyclosporine (Sandimmune) immunosuppression in patients receiving cadaveric kidney transplants (62,63). A total of 412 renal transplant patients were randomized to tacrolimus ($n = 205$) or cyclosporine ($n = 207$). One-year patient survival rates were 95.6% for tacrolimus and 96.6% for cyclosporine ($P = 0.576$) and 1-year graft survival rates were 91.2% and 87.9%, respectively ($P = 0.289$). Of note, biopsy-confirmed acute rejection was significantly reduced for tacrolimus patients (30.7%) compared with cyclosporine (46.4%, $P = 0.001$), and the requirement for antilymphocyte therapy for rejection was also significantly less for tacrolimus (10.7% and 25.1%, respectively; $P < 0.001$). Impaired renal function, gastrointestinal disorders, and neurologic complications, which were rarely treatment limiting, were common in both treatment regimens. Tremor and paresthesia were more frequent for tacrolimus, and the incidence of post-transplant diabetes mellitus was 19.9% for tacrolimus patients versus 4.0% for cyclosporine ($P < 0.001$), but it was reversible in some patients. Hyperlipidemia and hypercholesterolemia were decreased in the tacrolimus group, and hirsutism and gum hypertrophy were rarely seen, resulting in a greater improvement in health-related quality of life for these patients (64).

The results of this study were very similar to those observed in the European multicenter, randomized trial that compared the 12-month efficacy and safety of tacrolimus and cyclosporine (65). In that trial, a total of 448 renal transplant recipients from 15 centers received triple-drug therapy consisting of tacrolimus ($n = 303$) or cyclosporine ($n = 145$) in conjunction with azathioprine and low-dose corticosteroids. Twelve months after transplantation, tacrolimus therapy exhibited a significant reduction in the frequency of both acute rejection (tacrolimus 25.9% versus cyclosporine 45.7%; $P < 0.001$) and corticosteroid-resistant rejection (11.3% versus 21.6%, respectively; $P = 0.001$). Actuarial 1-year patient (tacrolimus 93.0% versus cyclosporine 96.5%; $P = 0.140$) and graft survival rates (82.5% versus 86.2%, respectively; $P = 0.380$) did not differ significantly between the two treatment groups. The safety profiles of the tacrolimus- and cyclosporine-based regimens were found to be comparable. No cost comparison results of either of these randomized studies have been reported.

In another randomized, prospective, single center trial of 395 adult patients undergoing renal transplantation, the effect of adding azathioprine to tacrolimus and steroids was evaluated (66). The 2-year actuarial patient and graft survival rates were 95% and 83%, respectively, with tacrolimus-based immunosuppression. There was no significant advantage in either patient or graft survival for patients given azathio-

prine, but the incidence of acute rejection was significantly higher with double than with triple therapy (54% versus 44%; $P < 0.05$).

Tacrolimus was also evaluated as rescue therapy in 73 patients with biopsy-confirmed renal allograft rejection (67,68). The median time to tacrolimus rescue therapy was 2.5 months (range 18 days to 48 months). At least one course of antilymphocyte therapy had been used in 59 (81%) patients prior to rescue. Responses to tacrolimus therapy included improvement in 78% of patients, stabilization in 11%, and progressive deterioration in 11%. Actuarial patient and graft survival rates were 93% and 75%, respectively, 12 months after initiation of tacrolimus therapy.

In a more recent report, the results of tacrolimus rescue therapy in 169 patients experiencing biopsy-confirmed rejection were summarized (69). The median time to rescue therapy was 2 months (range 2 days to 55 months), and antilymphocyte preparations had been used in 144 (85%) patients prior to rescue. Of the 144 patients unsuccessfully treated with antilymphocyte drugs, 117 (81%) were successfully rescued with tacrolimus. Twenty-eight patients were on dialysis at the time of conversion, and of these 13 (46%) were successfully rescued. In total, 125 (74%) patients were successfully rescued with tacrolimus and had functioning grafts at a mean follow-up of 30 months. Mean prednisone doses decreased from 28.0 ± 9.0 mg/day pre-tacrolimus to 8.5 ± 4.1 mg/day post-tacrolimus.

Of note, successful renal allograft function has been achieved with the addition of tacrolimus therapy even after the patient had been returned to dialysis, sometimes for prolonged periods (70). A review of these and other trials evaluating tacrolimus in renal allograft recipients has been recently published (71).

Kidney–Pancreas

Twenty-three recipients of kidney–pancreas transplants were studied to determine the value of tacrolimus primary immunosuppression in these patients (72). Of the evaluable patients, 10 received cyclosporine and 10 received tacrolimus therapy. The patient survival rate at 1 year was 100% for both the cyclosporine and the tacrolimus group, and the kidney graft survival rate was 90% for both groups. The pancreas graft survival rate was 80% for the tacrolimus group and 100% for the cyclosporine group (not significant). The time to the first rejection episode was significantly longer for tacrolimus compared to cyclosporine (23 versus 12 days, respectively; $P = 0.026$). There were no significant differences in the incidences of infectious complications or drug-induced nephrotoxicity.

Several other studies, with limited patient populations, have addressed the use of tacrolimus induction and rescue therapy after kidney–pancreas transplantation. In one study, the therapeutic effects of tacrolimus were documented in 61 kidney–pancreas or pancreas-only transplant patients with relapsing and resistant cellular rejection as well as chronic

vascular rejection after they had been switched from cyclosporine-based immunosuppression (73). A subsequent follow-up analysis of 166 patients receiving primary tacrolimus therapy revealed a one-year graft survival rate of 88% (74). Similarly, 10 kidney-pancreas transplant patients and 1 pancreas-after-kidney patient were converted to tacrolimus following acute severe cyclosporine nephrotoxicity (75). All 11 patients were alive and 10 combined grafts were still functional 7.7 months after tacrolimus rescue therapy. All of the patients maintained stable renal function and blood glucose levels after the conversion from cyclosporine to tacrolimus. These observations have now encouraged many transplant centers to adopt tacrolimus-based primary immunosuppressive regimens for pancreas-kidney transplant recipients (76) (see also Chapter 21).

Intestinal

Because of the relatively small number of procedures performed, data are more limited on the use of tacrolimus as immunosuppression following intestinal transplantation. Nevertheless, a few reports are available summarizing the results in both adults and children. In an initial report, tacrolimus was used as primary immunosuppression in 12 adults and 11 children who underwent small bowel, small bowel and liver, or multivisceral transplants (77). At a minimum follow-up of 2 months, 19 of 23 patients were alive. In a follow-up that included 15 small bowel recipients from the earlier report, patient and graft survival were 70% and 66%, respectively, at 18 months after transplant (78). This experience, now expanded to 98 consecutive recipients of intestine, liver and intestine, or multivisceral allografts, was recently updated to reveal actuarial 1- and 5-year survival rates of 72% and 48%, respectively (79). As discussed in Chapter 22, these and other reports have concluded that tacrolimus-based immunosuppression provides a higher survival rate following intestinal transplantation than any other currently available regimen.

Heart-Lung

In an open-label study, tacrolimus was evaluated as primary immunosuppression in 62 adult heart transplant recipients and as rescue therapy in an additional 10 patients (80). At a mean follow-up of 1 year, patient survival was 92%. The mean number of rejection episodes was 0.95 per patient during the first 90 days, and recurrent rejection occurred in 28% of patients. Among the 10 patients receiving rescue therapy, 7 remained free from rejection on tacrolimus. In another study of tacrolimus rescue therapy for 16 heart and 15 lung recipients suffering acute or humoral rejection while on cyclosporine, patient survival rates of 100% in the heart recipients and 67% in the lung recipients were achieved (81).

Tacrolimus was used as primary immunosuppression in 26 pediatric heart transplant recipients (82). Patient survival

was 82% at 1 and at 3 years, and 60% of patients were rejection-free at 3 and 6 months after transplantation.

Seventy-four adult patients were randomly assigned to tacrolimus or cyclosporine following single or bilateral lung transplant (83). One-year patient survival was similar between groups, but the number of patients free from acute rejection was significantly higher ($P < 0.05$) in the tacrolimus group. One patient (3%) in the cyclosporine group versus 5 of 38 (13%) in the tacrolimus group remained free from acute rejection during the first 120 days after transplant ($P < 0.05$). More patients in the cyclosporine group experienced bacterial infection, which was the major cause of late graft failure in both groups. A follow-up report of 133 patients reported similar 2-year survival rates for tacrolimus and cyclosporine but with a trend toward lower acute rejection rates with tacrolimus (84). Thirteen cyclosporine-treated patients versus 2 tacrolimus-treated patients required crossover to the alternative therapy ($P = 0.02$). The incidence of obliterative bronchiolitis was significantly lower with tacrolimus ($P = 0.025$).

Tacrolimus and cyclosporine were evaluated as primary immunosuppression in 20 pediatric lung transplant patients (85). Eight patients on each drug survived at a mean follow-up of 2 years after transplant. No differences in the incidence of rejection were reported between groups; however, 6 of 7 cyclosporine-treated patients and no tacrolimus-treated patients required antilymphocyte globulin for graft rejection. Furthermore, 4 patients on cyclosporine versus none on tacrolimus developed hypertension.

NONTRANSPLANT USES OF TACROLIMUS

Because of its potent immunosuppressive properties, tacrolimus may have applications in the treatment of autoimmune diseases (see Table 9.3). Clinical experience with tacrolimus has been reported in patients with nephrotic syndrome, psoriasis, chronic inflammatory diseases of the liver, and uveitis (86-90). Seven patients with refractory psoriasis experienced resolution confirmed with skin biopsy following treatment with tacrolimus (87). A marked reduction in proteinuria was observed in 6 of 7 patients with nephrotic syndrome, and the response was sustained for 6 months (86).

Ten patients with primary sclerosing cholangitis were treated with tacrolimus for up to 1 year (84). Serum bilirubin, alkaline phosphatase, and transaminase levels were reduced by 70% to 86%. No adverse effects on serum creatinine or blood urea nitrogen were observed with tacrolimus. In a second trial, 21 patients with autoimmune chronic active hepatitis were treated with tacrolimus for up to 3 months (90). Transaminase levels were reduced by 70% to 80%, with minimal increases in serum creatinine and blood urea nitrogen levels.

Tacrolimus was used to treat 13 patients with refractory uveitis and produced improvement in visual acuity during 6 weeks of follow-up (91).

Tacrolimus also has been investigated for prevention of graft-versus-host disease in patients undergoing bone marrow transplantation (92). In this pilot study, 18 patients were randomized to tacrolimus alone or with methotrexate or methylprednisolone. Eight of 18 patients developed grade II–IV acute graft-versus-host disease, and 1-year disease-free survival was 39%.

SAFETY/TOLERABILITY PROFILE OF TACROLIMUS

Much of the early toxicity reported with tacrolimus was associated with the use of unnecessarily high intravenous doses. The most frequent adverse events with tacrolimus in clinical trials have been renal impairment (highlighting the lack of correlation with blood levels), abnormalities in glucose metabolism, and neurotoxicity (Table 9.4) (55,56). In both of the liver transplant multicenter trials, the incidence of abnormal kidney function in the early post-transplant period was significantly increased with tacrolimus compared with the cyclosporine group. However, serum creatinine concentrations were not significantly different between treatment groups at the 12-month follow-up. The decrease in renal toxicity over time presumably reflected a decrease in the dose of tacrolimus and a more rapid conversion to oral therapy as experience with tacrolimus increased.

A randomized, prospective study on the effects of tacrolimus immunosuppression on lipid profiles in stable cyclosporine-treated renal transplant patients with established hyperlipidemia was undertaken by the Southeastern Organ Procurement Foundation (93). Patients with cholesterol of 240 mg/dL or greater, who were at least 1 year post-transplant with stable renal function, were randomly assigned

to remain on cyclosporine (control) or be converted to tacrolimus. Renal function, glucose control, and levels of total cholesterol, triglycerides, total high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and apoproteins A and B were monitored before conversion to tacrolimus. A total of 53 patients were analyzed (27 in the tacrolimus group and 26 controls) 6 months after conversion. In patients converted to tacrolimus treatment, there was a significant decrease in cholesterol (16%; $P = 0.0031$), LDL cholesterol (25%; $P = 0.0014$), and apolipoprotein B (23%; $P = 0.034$). There was no change in renal function, glycemic control, or incidence of new onset diabetes mellitus in the tacrolimus group. The authors concluded that conversion to tacrolimus from cyclosporine should be considered in the treatment of post-transplant hyperlipidemia.

New onset diabetes mellitus and hyperglycemia occurred significantly more often with tacrolimus in both the US and the European trial (55,56). Of concern is that many patients developed de novo insulin-dependent diabetes mellitus several months after transplantation, long after high-dose steroid therapy was given. However, later studies combining tacrolimus with mycophenolate mofetil (MMF) suppression indicate the incidence of insulin-dependent diabetes is reduced to 10% or less (94). The incidence of neurologic adverse events, including tremor and paresthesia, was significantly higher with tacrolimus, but the events were generally of mild or moderate severity. However, severe neurologic adverse events—such as convulsions, confusion, psychosis, encephalopathy, and even coma—were also seen, and they responded to a reduction in tacrolimus dosage. In the European trial, a decreased incidence of infection was reported with tacrolimus, while in the US study the incidence of both malignant and nonmalignant neoplasms was lower with tacrolimus. In a 2-year follow-up of the US study, a higher incidence of post-transplant hepatitis C was reported in the tacrolimus group, and the incidence of rejection was higher in this subpopulation of tacrolimus-treated patients. Whether hepatitis C is a more serious problem in those receiving tacrolimus than in those receiving cyclosporine is presently being debated (95,96).

Pediatric patients receiving tacrolimus following liver transplantation have experienced an increased incidence of neurotoxicity, diarrhea, and dyspepsia; however, the incidence of infectious complications was similar to that with cyclosporine (97). In contrast, a higher incidence of viral infections was reported in pediatric patients undergoing kidney transplantation (70). Of particular concern is the high incidence of lymphoproliferative disease, which may approach 10% in the pediatric population (98,99), and the occasional development of red cell aplasia (100) or hemolytic uremia (101). Because a large proportion of children are Epstein-Barr virus (EBV) seronegative and, thus, at risk for primary infection leading to an increased risk of post-transplant lymphoproliferative disease, a role for EBV prophylaxis needs to be clarified (see also Chapter 20).

Table 9.4. Common Adverse Events with Tacrolimus

Gastrointestinal
Anorexia
Diarrhea
Dyspepsia
Nausea
Metabolic
Hyperglycemia
Hyperkalemia
Nephrotoxicity
Neurologic
Confusion
Encephalopathy
Headache
Paresthesia
Psychosis
Seizure
Tremor

DOSING AND ADMINISTRATION

Because of good intestinal absorption, intravenous administration of tacrolimus is generally not required in the immediate postoperative period, when tacrolimus can be administered through the nasogastric tube. The initial dose of tacrolimus is 0.05 mg/kg every 12 hours, with subsequent dosage adjustment depending on the measured blood levels. The targeted tacrolimus whole blood level is initially 10 to 20 ng/mL. After 4 weeks the recommended trough level is 5 to 15 ng/mL. The optimal long-term trough level has not yet been well defined. However, in many patients with stable allograft function we see tacrolimus trough levels measuring less than 5 ng/mL, which is below the reliable threshold of the current assay. With improved monitoring techniques, the therapeutic requirements should be better defined in the future.

Pediatric patients have higher clearance and larger volumes of distribution than adult patients and thus require at least two and sometimes three daily full oral doses of tacrolimus to maintain adequate whole blood concentrations (58). Also, pediatric patients are immunologically more active and often require higher drug levels to prevent rejection. Generally, intravenous administration is only required in patients who experience gut complications after transplantation. In those circumstances, the tacrolimus dose is 0.01–0.05 mg/kg per day by continuous infusion. In patients with impaired hepatic function or in patients with stage III–IV encephalopathy, tacrolimus should be initially withheld and an induction protocol using antilymphocyte antibodies considered (see Chapter 13). Also, in patients receiving suboptimal donor organs the tacrolimus starting dose should be reduced.

It should be recognized that the bioavailability of tacrolimus is highly variable, and ordinary doses can quickly make a patient profoundly toxic. Thus, careful monitoring is mandatory in all cases following initiation of therapy. Some patients require an inordinate amount of tacrolimus to produce therapeutic levels (e.g., 0.2–0.3 mg/kg twice daily). In such patients, ketoconazole, itraconazole, or diltiazem have been used effectively to inhibit the metabolism of tacrolimus and allow normalization of the dose requirements. This can be accomplished without any side effects from the antifungal medication.

During initiation of tacrolimus therapy and before stable tissue levels have been achieved, large dosage changes often are necessary. Generally, a 30% to 40% change in dosage achieves the desired results. With stable, well-maintained patients, when fine tuning is desired, small dosage adjustments of 1 mg at a time are appropriate. In patients whose tacrolimus levels are quickly escalating—for example, during periods of sudden deterioration of liver function—the tacrolimus dose should be entirely withheld until nontoxic levels have been restored. This can sometimes take several days, depending on the extent and degree of liver failure. It takes 5 half-lives of any drug before a new steady state has

been achieved, and in patients with advanced liver failure, the half-life of tacrolimus could easily reach 60 hours.

The most difficult side effect to manage with tacrolimus is minor neurotoxicity, especially in elderly patients. Minor neurotoxicity can be subtle, and the physician may not notice any abnormal behavior on casual examination. At times, a detailed interview of family and friends may be necessary to identify the cause. This toxicity can occur even with low therapeutic levels of tacrolimus, and its existence may warrant a further dosage decrease, even with blood tacrolimus levels of 5 ng/mL or less. If tacrolimus is not tolerated, one can always switch to cyclosporine. Although cyclosporine also causes neurotoxicity, the signs and symptoms are often different and thus may be relieved by using the alternate drug.

The role of triple therapy in tacrolimus protocols has not been extensively investigated. However, it is clear that the addition of azathioprine and MMF to the immunosuppressive regimen can be beneficial in tacrolimus-treated patients and may limit troublesome toxicity (94). However, with potent drugs such as tacrolimus and mycophenolate mofetil, one of the most common side effects in immunosuppressed patients is overimmunosuppression, which leads to infections and malignancies. Thus, initiation of a triple drug regimen must be done with utmost care.

COMMENTARY

Thomas E. Starzl and John J. Fung

Klintmalm's chapter contains useful information, the exploitation of which requires an understanding of the management principles that have guided organ transplantation since its inception. Only four drugs have been widely used as baseline immunosuppressants: azathioprine, cyclophosphamide, cyclosporine, and tacrolimus. With each agent, the dosage must be determined individually for every patient. The ceilings are imposed by toxicity, and the dose floors are revealed by breakthrough rejection. The amount of azathioprine and cyclophosphamide that can be given is limited by myelotoxicity, which can be monitored conveniently with serial white blood counts. The more complex side effects of cyclosporine and tacrolimus are shown in Table 9C.1.

HISTORICAL PERSPECTIVE

None of the four baseline drugs can reliably prevent post-transplant rejection when used alone. However, it was learned in 1962 to 1963 that organ rejection, which previously had been considered inexorable, could be reversed and that subsequent dose requirements of immunosuppressive agents frequently declined. The same events have been observed with all treatment regimens and with all organs. Delineation of this pattern of con-

valescence, which was first observed with the combination of azathioprine and prednisone (1), provided the empirical launching pad for the clinical field of organ transplantation, in which dose-maneuverable prednisone has proved to be the indispensable ingredient.

The introduction of each new drug was accompanied by an easily learned trial and potential error determina-

tion of the requisite doses of the individual constituents of the cocktail. With increasingly potent baseline agents, survival of all organ grafts rose in three distinct leaps over a 33-year period. The characteristic immunologic confrontation and resolution process did not change—it merely became easier to manage.

What was being accomplished through this process remained enigmatic until it was discovered in 1992 that long-surviving organ recipients had donor leukocyte chimerism in their blood, skin, lymph nodes, and other sites as late as three decades after transplantation. It was then obvious that the prototypic post-transplant phenomena were the product of a double immune reaction: host-versus-graft (rejection) and graft-versus-host (Fig. 9C.1). Potentially tolerogenic “passenger leukocytes” of bone marrow origin, including pluripotent stem cells, had migrated from organs and engrafted peripherally. This was the seminal mechanism of organ allograft acceptance (2).

The Tacrolimus Pilot Trials

The clinical development of tacrolimus bracketed the chimerism discoveries, beginning 3 years before and continuing for 5 years after. In 1989, we first showed that tacrolimus (FK506) could systematically reverse liver allograft rejection that had been intractable in the face of maximal cyclosporine-based conventional immunosuppression (3,4). The “rescued” patients were maintained thereafter on tacrolimus, and manifested no unique or unexpected toxicity (5). Consequently, a nonrandomized trial was begun in which tacrolimus was substituted for cyclosporine from the time of operation.

Table 9C.1. Nonimmunologic Profile (4+ = Worst), All Dose-Related

	Tacrolimus	Cyclosporine
Nephrotoxicity	++*	++
Neurotoxicity	+	+
Diabetogenicity	+	+
Growth effects		
Hirsutism	0	+++
Gingival hyperplasia	0	++
Facial brutalization	0	+
Hepatotropic effects	++++	+++
Gynecomastia	0	+
Other metabolic effects		
Cholesterol increase	0	++
Uric acid increase	+?	++

* Less hypertension.

Sources: From Starzl TE, Abu-Elmagd K, Tzakis A, et al. Selected topics on FK 506: with special references to rescue of extrahepatic whole organ grafts, transplantation of “forbidden organs,” side effects, mechanisms, and practical pharmacokinetics. *Transplant Proc* 1991;23:914–919. (Slide presented at the Transplantation Society Congress, San Francisco, in August, 1990.)

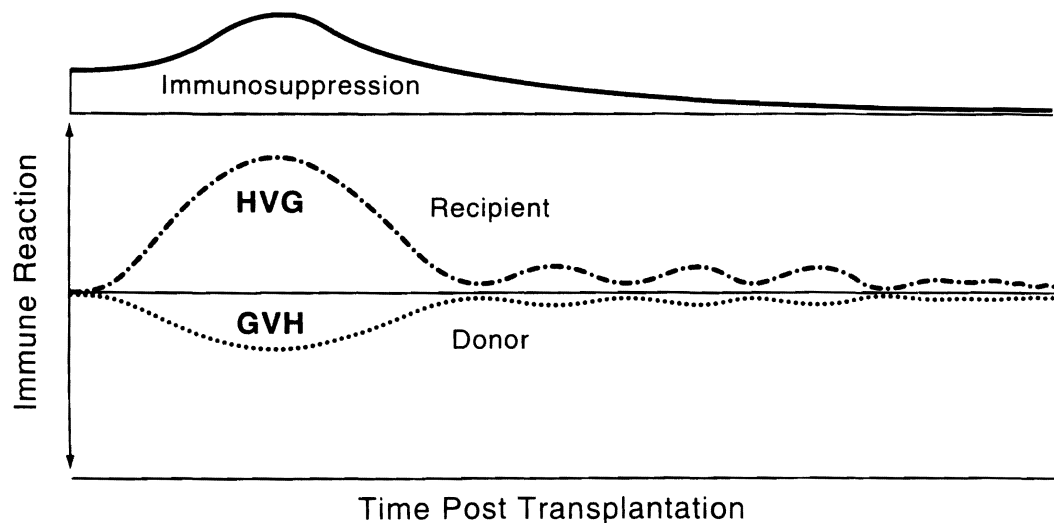


FIGURE 9C.1. Contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) reactions in the two-way paradigm of transplantation immunology (2). Following the initial interaction, the evolution of nonreactivity of each leukocyte population to the other is seen as a predominantly low-grade stimulatory state that may wax and wane, rather than as a deletional one.

By early 1990, nearly 200 liver, kidney, and other-organ recipients who had been entered in the program had superior actuarial survival, lower requirement for prednisone, and better quality of life than we had observed in the past (6–8). Already the upgrading of outlook after liver transplantation was as obvious as it had been a decade before when cyclosporine succeeded azathioprine as the baseline immunosuppressant (Fig. 9C.2). Thus, even as the advent of cyclosporine had elevated transplantation of cadaver kidneys from a previously unacceptable level (9,10), the bar rose for liver and for kidney transplantation with the introduction of tacrolimus (Fig. 9C.3). The same was true for thoracic organs (8).

It was clear by early 1990 that the dose-limiting side effects of cyclosporine and tacrolimus were the same: nephrotoxicity, neurotoxicity, and diabetogenicity (see Table 9C.1). These manifestations could be used from the first day of treatment to determine appropriate doses (6–8,11,12). Invidious toxicity comparisons between

cyclosporine and tacrolimus were unwarranted because the scales could be tilted one way or the other by ratcheting the doses up or down. The only adverse effects observed exclusively with one drug but not the other were the dose-related cosmetic changes caused by cyclosporine (see Table 9C.1).

As had been found a decade earlier with cyclosporine (11), it was easy to relate toxic manifestations and rejection to trough plasma and blood concentrations (the plasma/blood ratio of tacrolimus was about 1:10) and promptly endow the laboratory results with clinically relevant meaning (6–8,12,13). Flexibility of dosing was important no matter what the transplanted organ, but it was especially so with the liver because the metabolism of tacrolimus is more dependent than that of cyclosporine on good hepatic function (12,14). By the beginning of 1990, doses and trough levels used for liver, kidney, heart, and lung recipients in Pittsburgh (6,7) were essentially the same as those recommended in

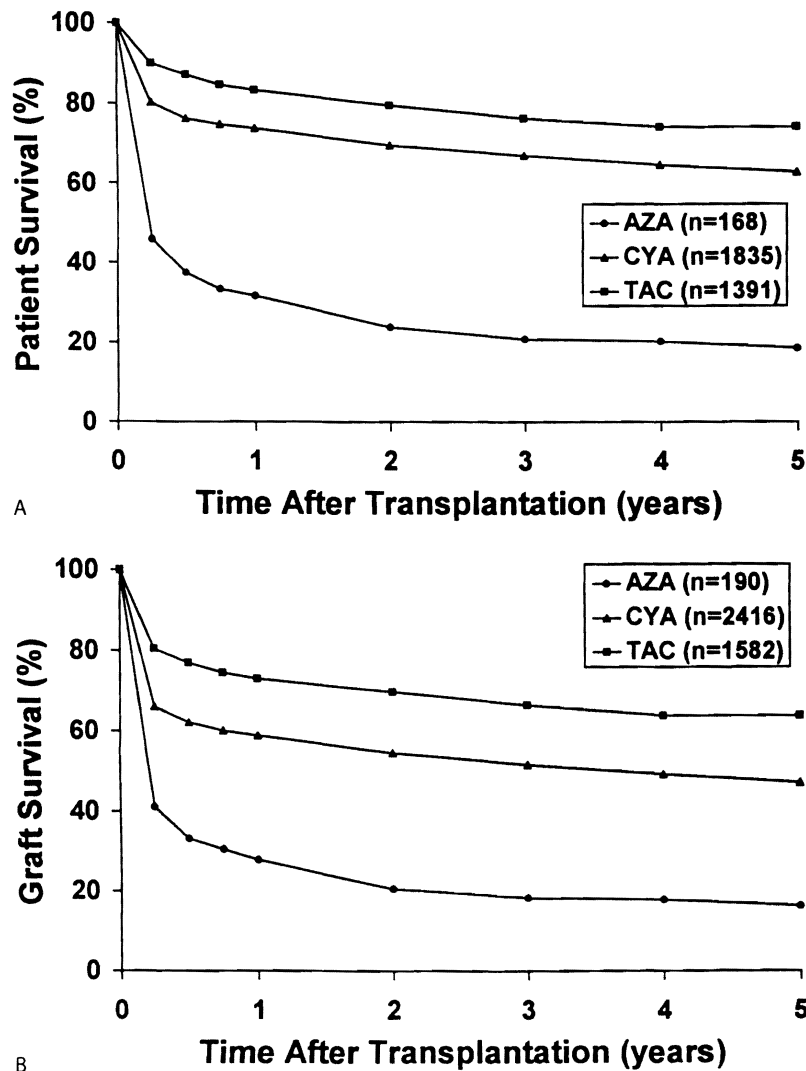


FIGURE 9C.2. The three eras of orthotopic liver transplantation at the Universities of Colorado (1963–1980) and Pittsburgh (1981–1993), defined by azathioprine, cyclosporine, and tacrolimus (FK 506) immunosuppression. **A.** Patient survival. **B.** Graft survival. This was about 10% lower than patient survival in both the cyclosporine era (1980–1989) and the tacrolimus era (1989–1993), whereas patient and graft survival were essentially identical with azathioprine. The difference in later series was explained by effective retransplantation, an option that did not exist previously. AZA, azathioprine; CYA, cyclosporine; TAC, tacrolimus.

Klintmalm's chapter. The data shown in Figure 9C.4 were presented on April 5, 1990, at the American Surgical Association, and were published 4 months later.

The Randomized Liver Trials

Historically, new immunosuppressive drugs were evaluated in kidney recipients and then applied secondarily to transplantation of unpaired vital organs. This precedent was broken with the development of tacrolimus, largely because liver transplant surgeons demanded that the drug be released for rescue therapy of their patients. Rather than pursuing the question of rescue efficacy, it was decided at meetings with the United States Food and

Drug Administration (FDA) during October and November of 1989 to proceed with randomized European and American multicenter trials comparing tacrolimus with cyclosporine as the primary immunosuppressant from the time of liver replacement. By the time these trials started the following autumn, however, a decisive trial that had started in February 1990 in Pittsburgh using the tacrolimus doses shown in Figure 9C.4 was more than half completed.

The Pittsburgh Liver Trial

The Pittsburgh Liver Trial was a single-center, Institutional Review Board (IRB)-mandated trial. Safety and

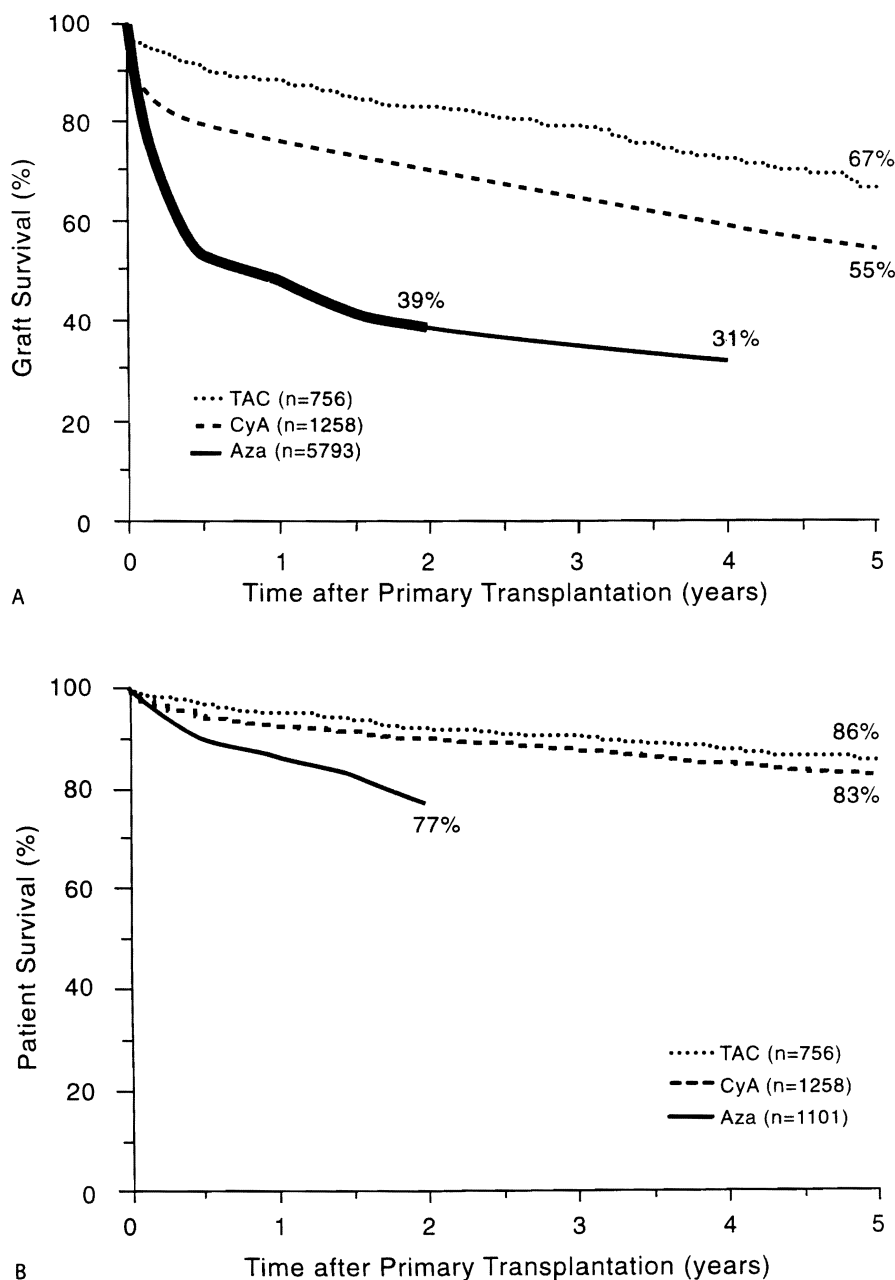


FIGURE 9C.3. (A) Patient survival and (B) graft survival after primary cadaveric kidney transplantation at the University of Pittsburgh under cyclosporine (1981–1989) versus tacrolimus-based immunosuppression. These observations suggest the long-term survival of kidney allografts can also be improved by substitution of tacrolimus for cyclosporine. Graft survival curves for azathioprine in A are from Terasaki's national register. Early patient survival data are from the Southeastern Organ Procurement Foundation, to which Pittsburgh contributed. TAC, tacrolimus; CyA, cyclosporine; Aza, azathioprine.

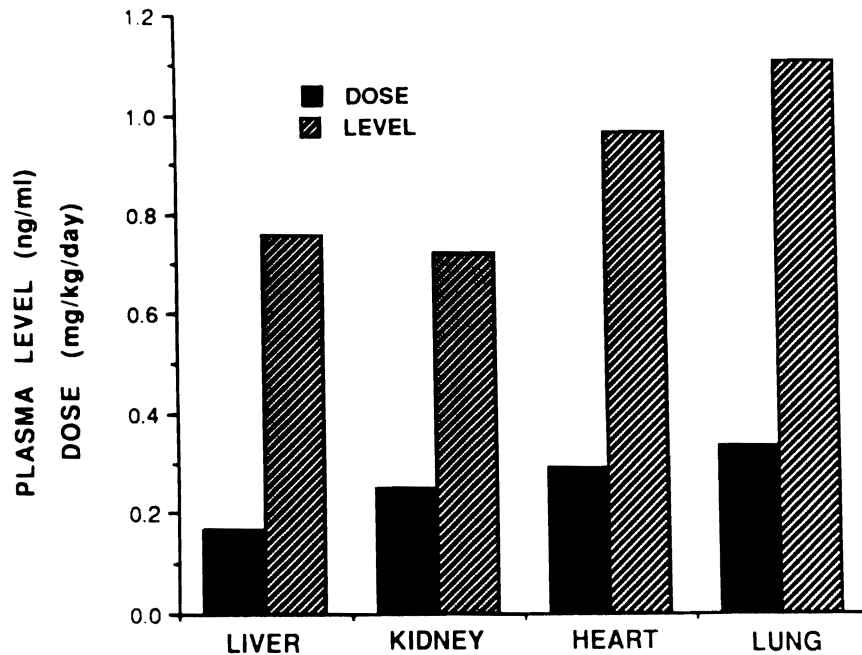


FIGURE 9C.4. The tacrolimus doses used in the Pittsburgh Liver Trial and initially recommended for recipients of other organs. Because of the dependence on good hepatic function for tacrolimus metabolism, starting doses for liver recipients are slightly lower.

Pittsburgh Protocol

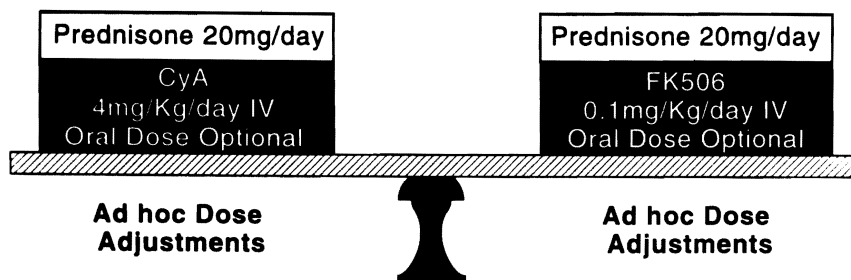


FIGURE 9C.5. The Pittsburgh Liver Trial protocol, tacrolimus (FK506) versus cyclosporine (CyA). All treatment variables except for the competing drugs were equalized.

efficacy comparisons were ensured by equalization of all treatment variables except the competing drugs (15,16) (Fig. 9C.5). Definition of end points allowed the liver recipients early access to whichever drug had the better therapeutic margin. The trial was prematurely terminated in 1991 by a multi-institutional oversight committee, which had been insisted upon by the investigators. By the time the trial was stopped, a massive crossover from cyclosporine to tacrolimus had occurred, with only one crossover from tacrolimus to cyclosporine. Throughout the entire 5-year period of subsequent study, tacrolimus enjoyed a statistically significant greater freedom from rejection, either alone or in combination with freedom from graft loss and adverse events (16).

The Multicenter Liver Trials

The design of the subsequent American (17) and European (18) liver transplant trials, which involved 20 insti-

tutions, was of some concern. In all 20 participating centers, the cyclosporine arm was up-loaded with twice (or more) the induction doses of prednisone used for the tacrolimus patients. The cyclosporine-treated recipients also were given a third drug (azathioprine) in 95% of the centers and a fourth agent (polyclonal ALG) in a few (Fig. 9C.6). The tacrolimus doses were set higher than those concurrently used in Pittsburgh, and they remained so until the end of the study (Fig. 9C.7). The combination of excessive dosage and the sometimes delayed response to toxic events on the tacrolimus arm complicated the interpretation of some observations.

The European teams had a 5% better survival of patients on the tacrolimus arm [17% (46/270) versus 22% (61/275) mortality] and a 5% higher graft survival (18). By intent-to-treat analysis, the survival advantage was not statistically significant. However, about 10% of the surviving grafts credited to cyclosporine had been rescued from treatment failure with tacrolimus. The distorting

USA Protocols

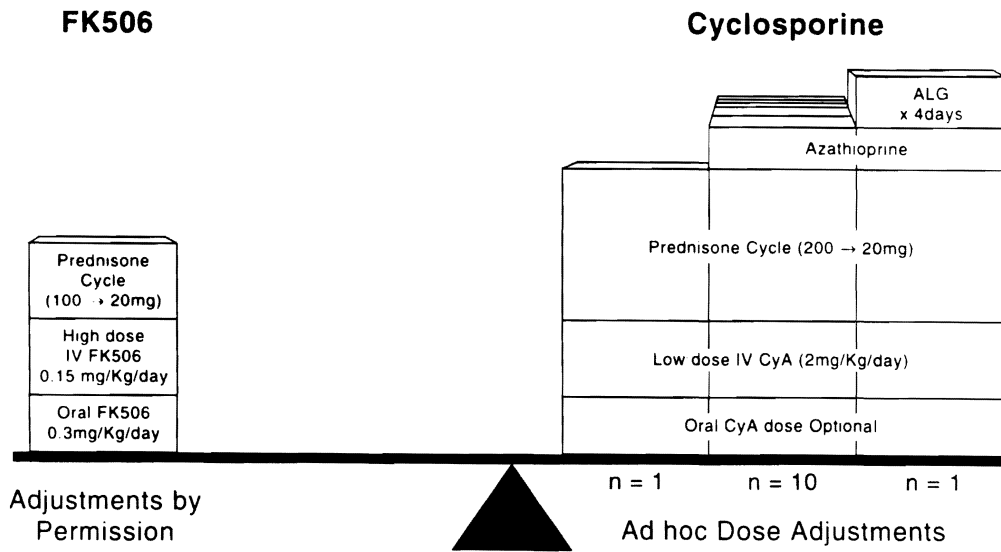


FIGURE 9C.6. US protocol in the multicenter tacrolimus (FK506) trial. The cyclosporine (CyA) arm was uploaded with twice (or more) the induction doses of prednisone used for the tacrolimus patients. Cyclosporine recipients also were given a third drug (azathioprine) in 95% of the centers; polyclonal ALG was used in a few centers (n, number of centers using the regimen).

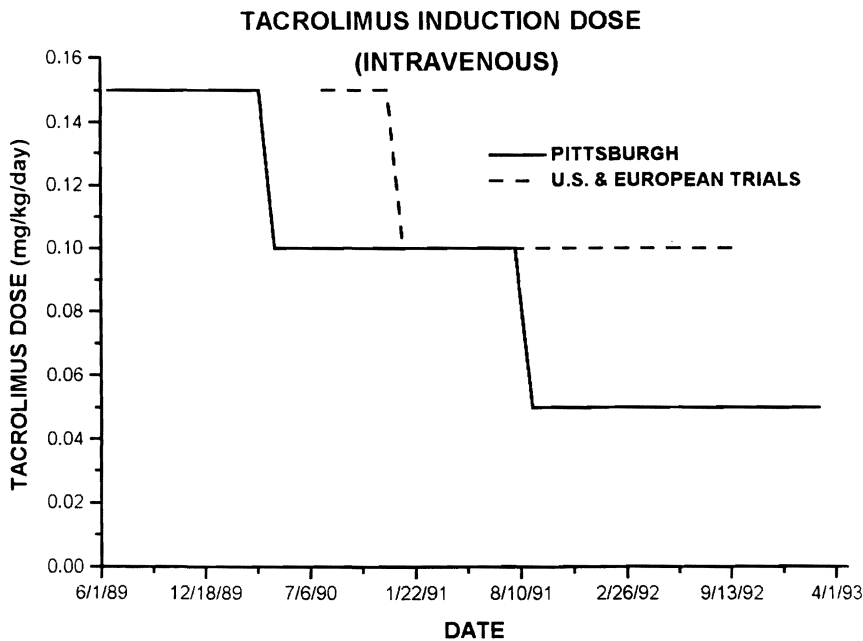


FIGURE 9C.7. Tacrolimus doses set for the Multicenter Liver Trial. The doses were higher than those used in the Pittsburgh study.

roles of tacrolimus overdosage and the consequent high rate of toxicity were clarified by separate analyses of the early (high dose) and later (reduced dose) phases of the trial. The statistical analysis, based on the intent-to-treat approach, showed significantly greater freedom from acute rejection, intractable acute rejection, and chronic rejection. The report concluded that tacrolimus had superior therapeutic qualities (18).

In the published American report (17), use of the Kaplan-Meier and intent-to-treat methods created the impression that the better efficacy and greater toxicity of tacrolimus essentially balanced each other. An alternative analysis suggested that freedom from rejection as a single end point was accomplished more frequently with tacrolimus (19). Moreover, the superiority of tacrolimus emerged at all levels, including freedom from adverse

events and, most significantly, freedom from refractory rejection. After 1 year of follow-up, 98% of the patients randomized to tacrolimus were not diagnosed with refractory rejection, compared with 87% in the competing arm. The composite freedom at 1 year from the three factors that haunt transplant recipients—refractory rejection, retransplantation, and death—was 80% for tacrolimus and 70% for cyclosporine (19).

Randomized Trialomania

Disquieting scientific and ethical issues were exposed by the events of the 6 years following the placement of tacrolimus on the FDA fast-track in November 1989. Neither the new drug's unusual rescue capability (3–5) nor its superiority as a baseline agent (6,7) were in doubt from late 1989 onward. The evidence for this as well as management recommendations had been published by the summer of 1990 (3,6–8,12), although, as with all new therapies, independent confirmation was needed from other centers. The FDA has a range of options for confirmation, as shown in Table 9C.2. However, the agency has increasingly insisted on multicenter, controlled, randomized trials as a prerequisite for marketing new drugs, and tacrolimus would be no different.

The FDA feared that pilot studies might suggest superiority of tacrolimus to potential multicenter participants, making them as reluctant as the Pittsburgh investigators had been to participate in a randomized trial. Therefore, no familiarization cases that used the drug from the time of transplantation were allowed. Also, by the nature of transplantation biology, cocktail regimens can never be applied in exactly the same way to any two recipients. In spite of this, the multicenter trial design insisted on rigidity of therapeutic protocols for tacrolimus (but not for the competing drug).

Although the design and dosing errors of the multicenter liver trials were ascribed to the investigators, the protocols and the trials themselves were efforts to comply with regulatory requirements. The FDA does not ostensibly engage in human experimentation; however, when

Table 9C.2. Spectrum of Most to Least "Adequate" Controls

1. Placebo concurrent control (randomized, double-blinded).
2. Dose-comparison concurrent control (randomized, 2 doses, often with placebo or active treatment arm [competing drug]).
3. No treatment concurrent control (randomized, with no treatment arm).
4. Active treatment concurrent control (randomized versus competing agent).
5. Historical control versus concurrent treatment (special situations, when outcome is self-evident or in absence of equipoise).

Code of Federal Regulations (CFR 314.126). Revised by FDA, April 1, 1994.

the agency imposed a learning curve for tacrolimus on 20 different centers as a condition for the sale of a new drug that was urgently needed for rescue purposes, the agency became the de facto instigator of a human experiment. It is not possible to pull this switch and disavow responsibility for what followed.

Ill-advised or poorly designed randomized trials achieve the opposite objectives of their intent: improved and less expensive patient care. The lack of familiarization studies, together with the inexplicable error in dosing and the disadvantage of no on-site assays for drug monitoring, resulted in the muddled picture of the new drug's potential that emerged in the published literature between 1992 and 1995. Nevertheless, tacrolimus survived the irrational gauntlet not only because it was user friendly, but also because the talented group of multicenter clinical investigators rebelled against the protocol and introduced treatment flexibility. This confirmed our earlier experience, that throughout the entire history of liver transplantation only two key developments have upgraded graft and patient survival: Calne's introduction of cyclosporine in 1979 (21) and its use in combination with prednisone (22), and the arrival of tacrolimus (see Fig. 9C.2).

Other-Organ Uses

Following the uneven literature that came out of the American multicenter liver trials, an avalanche of articles involving organ after organ, and eventually bone marrow transplantation, would confirm essentially every detail of the original Pittsburgh experience (20).

The same developmental leaps were seen in renal transplantation, for example. The 1-year cadaver kidney survival rate in the United States had remained fixed at less than 50% until the advent of cyclosporine, after which it rose to 77% at the University of Pittsburgh. A second abrupt increase to nearly 90% followed the routine use of tacrolimus (see Fig. 9C.3). The maintenance of the gap between cyclosporine and tacrolimus after 1 year was congruent with the report of Gjertson, Cecka, and Terasaki, based on the cadaver kidney half-life projections from 24 American kidney transplant centers with access to tacrolimus. Their suggestion was: "FK 506 (tacrolimus) appears to be the first therapeutic agent to significantly improve long-term kidney graft survival rates" (23). A thorough review of the use of tacrolimus following kidney transplantation has been provided by Laskow et al (24).

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

It has not been surprising to observe an incremental increase in post-transplant lymphoproliferative disorders (PTLDs) with the successively more potent baseline

immunosuppressants (21,25–27). This risk can be reduced at the outset by avoiding the joint use of the biologic antilymphoid agents (e.g., ALG and OKT-3) in conjunction with cyclosporine and tacrolimus except as a last resort, and then only with extreme caution.

When PTLD is diagnosed early in development, it usually is a trivial problem requiring only drug dose reduction. At the Children's Hospital of Pittsburgh, 9 (13.2%) of 68 recipients (of 69 kidney allografts) treated with tacrolimus-based immunosuppression between 1989 and 1995 developed histopathologically verified PTLD (28). No deaths resulted, nor did any graft losses. All kidneys are still functioning (at the time of this writing) except one that was chronically rejected 3 years later.

At the same institution, histopathologically verified PTLD was diagnosed in 28 (12.1%) of the 232 consecutive primary pediatric liver recipients treated with tacrolimus between 1989 and 1995. Although 5 of the 28 died of potentially PTLD-related complications, the 4-year patient and graft survival rate (82.2%) was essentially the same as in the 204 non-PTLD patients (Table 9C.3) (27). Management was facilitated by the policies of gradual tacrolimus dose reduction with acceptance of lower blood levels as time passes (see Table 9C.3), early discontinuance of prednisone, avoidance of adjunct agents including OKT-3 and azathioprine, and surveillance for Epstein-Barr virus (EBV) infection.

Table 9C.3. Primary Liver Transplantation in Children Under Tacrolimus (n = 232)^{a,b}

	Months' Follow-Up				
	3	12	24	36	48
Survival (%)					
Patient	90.2	86	85	84	84
Graft	83	79.2	78.2	77.3	77.3
Tacrolimus (mean)					
Dose (mg/day)	5.6	3.3	2.9	2.9	2.4
Plasma concentration (trough ng/mL) ^c	0.84	0.56	0.43	0.4	0.24
Prednisone (%)					
None	79	82	88	93	91
≤5 mg/day	9	13	7	5	7
>5 mg/day	12	5	5	2	2

^a September 1989–January 1995. Age 5.0 ± 5.2 (SD) years (medium 2.7). Mean follow-up 44 ± 14.6 months.

^b 28 patients developed PTLD. Principal sites were the gastrointestinal system (n = 9), lymph nodes (n = 8), liver (n = 6), spleen (n = 2), and tonsils, skin, and blood (leukemia) (n = 1 each).

^c For approximate whole blood values, multiply by 10.

Data from Dr. Jorge Reyes. Presented at the 2nd International Congress on Pediatric Transplantation, Paris, France, August 23–24, 1996.

Except for their frequent EBV association, human B-cell lymphomas are indistinguishable from those induced by Robert S. Schwartz in a mouse chimerism model (29) 3 years before they were reported in human kidney recipients (30). Schwartz attributed the experimental tumors to an active lymphoproliferative response by the dominant immune apparatus to the persistent subclinical graft-versus-host counterattack of the minority donor leukocyte population. The relevance of his observations to clinical PTLD would only be appreciated 30 years later (27), after the discovery that similar microchimerism was a characteristic feature of successful organ transplantation (2). This fresh insight about PTLD has been used to map treatment strategies of cellular immune modulation as discussed elsewhere (27).

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