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PERSPECTIVES IN RENAL MEDICINE

Rapamycin in transplantation: A review of the evidence

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Rapamycin in transplantation: A review of the evidence. The calcineurin inhibitors have been the mainstays of immunosuppression for solid organ transplantation over the last two decades, but nephrotoxicity limits their therapeutic benefit. Rapamycin is a new drug with both immunosuppressant and antiproliferative properties that has a unique mechanism of action distinct from that of the calcineurin inhibitors. It has a role as a maintenance immunosuppressant either alone or in combination with a calcineurin inhibitor and can also be used to treat refractory acute rejection. Theoretical evidence suggests that it may limit the development and progression of chronic rejection in transplant recipients, but this has yet to be confirmed. This review examines the current in vitro animal and human work underlying the use of rapamycin and, in addition, comments on the pharmacokinetics and side-effect profile of this promising new agent.

Rapamycin (Rapa, Rapamune, Sirolimus; Wyeth-Ayerst Pharmaceuticals, Philadelphia, PA, USA) is a macrocyclic fermentation product of *Streptomyces hygroscopicus*, an actinomycete, originally isolated from a soil sample in Rapa Nui (Easter Island, 1975) [1, 2]. Rapa was initially investigated as an antifungal and antitumor agent. However, its lymphopenic properties heralded its role as an immunosuppressant. There is currently much interest in Rapa because of its unique mechanism of action, lack of end-organ toxicity, and its ability to synergize with other immunosuppressants, yet avoid overlapping side effects. This review discusses the current evidence on which the use of Rapa in transplantation is based.

MECHANISM OF ACTION

The mainstays of modern immunosuppression, Cyclosporin (CsA) and Tacrolimus (FK506), bind to the intracellular cytosolic immunophilins cyclophilin and FK binding protein 12 (FKBP12), respectively, inhibiting calcineurin phosphatase. This prevents transcription of cytokines [for example, interleukin-2 (IL-2)] and progression of the T-cell cycle from G0 to G1 [3]. Rapa has a similar molecular structure to FK506 and also binds to FKBP12 [4]. However, the Rapa-FKBP12 complex has no effect on calcineurin phosphatase. Instead, it binds to one or more proteins known as "targets of rapamycin" (TOR) [5]. These effector proteins were originally demonstrated as TOR1 and TOR2 in yeast [6], but a mammalian homologue has now been identified [7]. This has been given a number of acronyms, including mTOR, FRAP, SEP, and RAFT1 [7–10], but "mammalian target of rapamycin" (mTOR) is most commonly used. Both cytokines, such as IL-2 and the CD28/B7 costimulatory pathway activate mTOR, resulting in downstream events critical for cell cycle regulation (Fig. 1). This process is complex and the underlying metabolic pathway has not been fully characterized [11–15]. However, the Rapa-FKBP12 complex binds mTOR and subsequently inhibits both DNA and protein synthesis, resulting in arrest of the cell cycle in late G1 as it progresses to the S phase [16].

IMMUNOSUPPRESSION

In vitro

The immunosuppressive properties of Rapa result from inhibition of leukocyte activity. It blocks T-cell proliferation induced by cytokines (IL-1, -2, -3, -4, -6, -7, -12, and -15), alloantigens, and mitogens in a dose-dependent manner [17–19]. However, Rapa does not appear to alter IL-2 induced T-cell apoptosis (Abstract 73; *Transplantation Society XVII*, World Congress, Montreal, Canada, 1998). Natural killer, cytokine-activated killer, and antibody-dependent cell cytotoxicity functions of human leukocytes are suppressed by Rapa at concentrations 10- to 100-fold greater than those needed to block T-cell proliferation [20]. Rapa acts on B cells independently of its effects on T helper cells, causing an inhibition of antigen and cytokine driven B-cell proliferation [21]. In addition, it has been shown to inhibit cytokine-dependent (IL-2 and IL-6) differentiation into antibody-producing cells, thus decreasing immunoglobulin synthesis [22].

In contrast to the calcineurin inhibitors, it has been claimed that Rapa has limited effects on cytokine expres-

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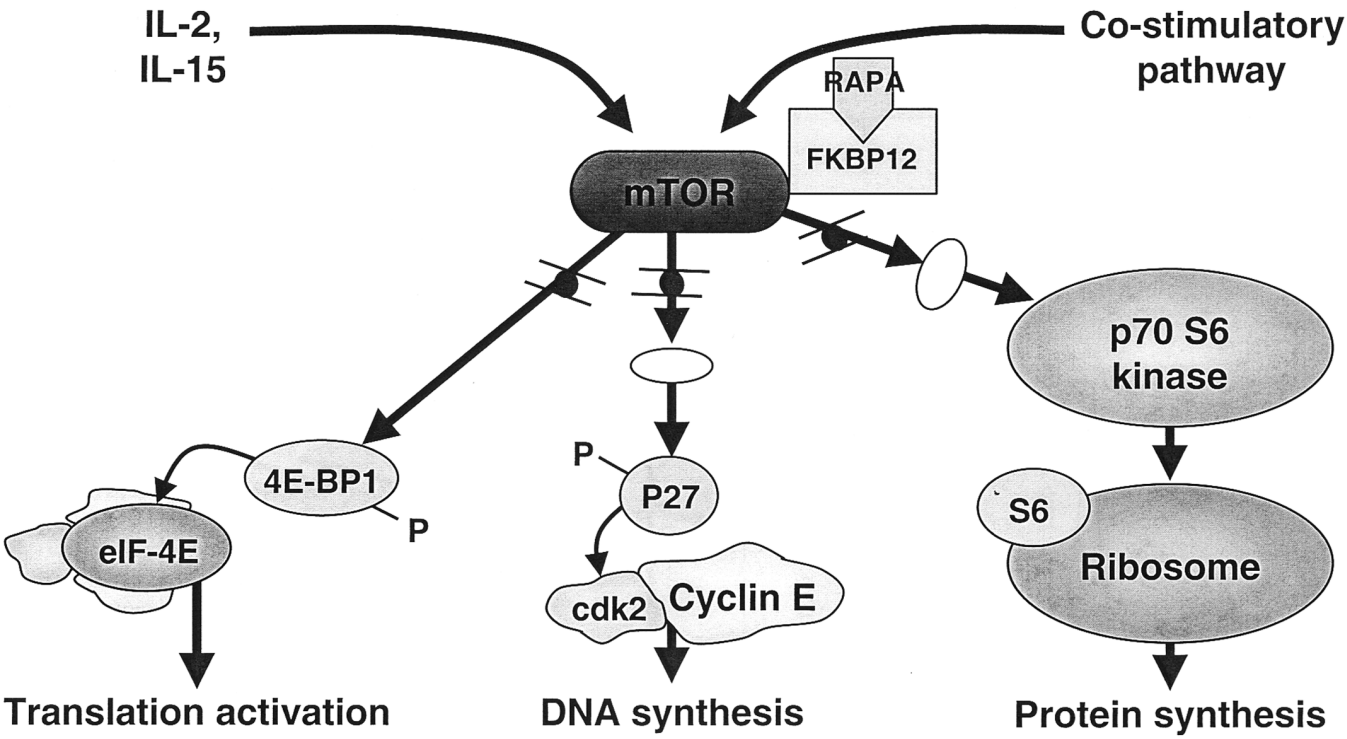

Fig. 1. Proposed mechanism of action for rapamycin. Abbreviations are: mTOR, mammalian target of rapamycin; IL, interleukin. Rapa inhibits (*1*) Phosphorylation of 4E-BP1, preventing release of eIF-4E and initiation of translation [12, 13]; (*2*) P27-mediated activation of cdk2-cyclin E and synthesis of proteins important for cell cycle progression [14]; and (*3*) p70S6 kinase activation, limiting ribosomal protein S6 phosphorylation and reducing synthesis of ribosomal/translational proteins [15, 16].

sion in vitro. It does not inhibit the transcription of IL-2, -3, -4 or tumor necrosis factor-α (TNF-α) in mitogen-activated T cells [17] and has been shown to have variable effects on interferon-γ (IFN-γ) transcription dependent on the stimulus [23]. However, work comparing the expression of TH1 and TH2 cytokines using reverse transcription-polymerase chain reaction (RT-PCR) in concavalin A-stimulated spleen cells suggested that Rapa inhibited the expression of IL-2, IFN-γ, IL-4, and IL-10 more than nonimmunosuppressed controls [24]. Indeed, Rapa inhibited IL-10 expression more effectively than CsA (100 vs. 65% inhibition). Rapa's main mode of action is the inhibition of cytokine-activated signal transduction, but clearly, any inhibitory effect on proinflammatory cytokine transcription (however minor) would complement its immunosuppressive efficacy. The underlying mechanism for this is not known but may stem from the inhibition of costimulatory pathway transduction as this has been implicated in the transcriptional activation of several cytokine genes.

The concept of inhibiting cytokine transcription and simultaneously blocking cytokine-mediated signal transduction has been investigated in vitro using CsA/Rapa combinations. Rapa augmented the inhibitory effects of CsA on antibody and phytohemaglutinin-stimulated peripheral blood leukocyte activation [25]. Furthermore, relatively high doses of Rapa and CsA alone were required to inhibit cell-mediated lympholysis in vitro (IC50 Rapa = 8721×10^{-9} M, CsA = 1052×10^{-9} M). However, the same degree of inhibition was produced by a combination of both at 72 and 144×10^{-9} M, respectively. In a further study, the IC50s required to inhibit the proliferation of an IL-2–dependent cell line for Rapa and CsA alone were 90.9 and 2602×10^{-9} M, respectively, compared with 65 and 260×10^{-9} M when combined [23]. This work has shown that the combination of Rapa and CsA is highly complimentary. Reduced doses of both drugs achieve a response much greater than one would expect from a purely additive effect, suggesting a synergistic interaction. The pharmacokinetic/pharmacodynamic mechanisms underlying this are not clear. However, this combination ensures that only small amounts of cytokine are produced, causing reduced activation of cytokine receptors and an attenuated downstream signal that is more easily inhibited by Rapa. It was hoped that the combination of FK506 and Rapa would display similar synergism. However, equimolar doses produced an additive effect, and if either was given in 50- to 1000-fold excess, they became antagonistic in vitro [26, 27].

Animal studies

The first in vivo studies documenting the immunosuppressive properties of Rapa were published by Calne et al

Table 1. Summary of the immunosuppressive efficacy of rapamycin alone at preventing graft loss secondary to the development of acute rejection in animal allografts

Species	Type of allograft [Reference]	Rapamycin dose *mg/kg/day*	Route of administration	Maximum duration of treatment *days post-op* unless stated	Mean number of days graft survival > controls
Mouse	Skin [30]	0.25–4	IP	6	4–5
	Heart [29]	0.75–3.0/6.0	IP/PO	14	15–136/5
Rat	Skin [31]	3.0 and 10.0	IM	8	6
	Heart [31]	0.1–5.0	IM	11	9–100+
	Heart [28]	0.5–5	IM	10	10–90
	Heart [33]	0.04	IV	14	7
	Heart [34]	0.16–0.8	IV	14	32–41
	Heart [32]	0.25	IP	7[a]	45
	Heart [35]	0.2–1.2	PO	30	13–55
	Kidney [33]	0.01–0.04	IV	14	10–100+
	Kidney [34]	0.8	IV	14	88
	Kidney [35]	0.2–0.4	PO	30	2–6.5
	Small bowel [34, 36]	0.8	IV	14	17
	Pancreas [35]	0.2–0.4	PO	30	5–8
	Pancreaticoduodenal [37]	0.8	IV	14	48
Rabbit	Heart [38]	0.05–1.0	IV	60	> control[c]
Dog	Kidney [31]	0.3–1.5	IM	Lifespan	2–10 < control[b]
	Kidney [39]	0.05	IV	7–20	16[b]
	Kidney [40]	2.0	PO	Days 3–5	1[b]
Pig	Kidney [41]	0.25	IM	30	53
	Kidney [40]	2	IM	64	65
	Kidney [42]	0.1–2.0	PO	28	4–24
Baboon	Kidney [43]	2–50	PO/IM/IV	15–20	0–30[b]

Abbreviations are: IP, intraperitoneal; PO, oral; IM, intramuscular; IV, intravenous.
[a] Preoperative administration only
[b] Severe gastrointestinal side effects
[c] Mean values not recorded

and Morris and Meiser [28, 29]. Both groups gave Rapa at various doses to rats receiving heterotopic cardiac allografts and noted longer graft and animal survival rates in comparison to nonimmunosuppressed controls. Similar benefits have been demonstrated for skin, renal, small bowel, pancreatic, and pancreaticoduodenal allografts in a number of species, including mice, rats, rabbits, pigs, dogs, and primates, although in dogs and primates fatal gastrointestinal side effects frequently occurred [28–43]. It is difficult to compare the immunosuppressive efficacy of Rapa between studies as various doses, routes of administration, and allograft models have been used (Table 1). However Rapa is 20 to 100 times more potent than CsA and acted in a dose-dependent manner to prevent acute allograft rejection. In studies that measured trough levels, a range of 5 to 10 ng/mL provided effective immunosuppression in small animals, but larger animals required levels >10 ng/mL. Subsequently, Rapa (0.8 mg/kg/day intravenously) was investigated as a potential treatment for ongoing acute rejection and was found to prolong the survival of presensitized rat skin and cardiac allograft recipients [44]. This dose-dependent effect (Rapa 0.08 to 0.8 mg/kg) was confirmed in cardiac, renal, and pancreas allografts with acute rejection [45, 46].

The synergy between Rapa and CsA suggested in vitro was also observed in animal work. Subtherapeutic doses of Rapa (0.01 to 0.04 mg/kg/day) and CsA (0.5 to 2.0 mg/kg/day) prolonged rat cardiac and kidney allograft survival compared with either drug alone or the additive effect of a combination of both [33]. Similar results were observed in studies using rat lung [47] and mouse kidney allografts [48] as well as a mongrel canine model [39].

In the preliminary investigation of Rapa (1991), a Rapa/FK506 combination acted synergistically in prolonging mouse heart tissue allograft survival [49]. However, in vitro studies contradicted these findings and discouraged investigators until relatively recently. In 1997, subtherapeutic doses of Rapa (0.02 to 0.04 mg/kg/day intravenously) and FK506 (0.01 to 0.04 mg/kg/day intramuscularly) in rat cardiac allografts not only prevented the development, but also reversed active acute rejection [50]. The therapeutic combination index used confirmed the efficacy of these agents with no evidence of the antagonism observed in vitro. Larger doses of oral Rapa (2 to 4 mg/kg/day) and intramuscular FK506 (2 to 4 mg/kg/day) demonstrated a similar extended survival in a mouse small bowel allograft model [51]. The contradictory findings in vitro and in vivo are difficult to reconcile. However, only a small fraction of FKBP12 needs to be occupied by either Rapa or FK506 in order to achieve maximal immunosuppression [52]. FKBP12 is abundant in vivo, and thus, competitive inhibition is unlikely to be a problem [53]. In vitro, on the other hand, it is limited, and the opposite applies. In addition, it is possible that these drugs have immunosuppressive mechanisms that

Table 2. A comparison of the results currently available from the ongoing multicenter trials

Multicentre trial and immunosuppression used	Rate of acute rejection (%)		One-year allograft survival	One-year patient survival	Two-year allograft survival	Two-year patient survival
	6 months	12 months				
US						
CsA/Aza/Pred (control)	24		93.8	98.1	91.3	96.9
CsA/Rapa 2 mg/Pred	12[a]		94.7	97.2	90.2	95.4
CsA/Rapa 5 mg/Pred	10[a]		92.7	96.0	90.1	94.9
Global						
CsA/Placebo/Pred (control)	41.5		87.7	94.6	86.1	93.1
CsA/Rapa 2 mg/Pred	24.7[a]		89.9	96.5	85.5	93.0
CsA/Rapa 5 mg/Pred	19.2[a]		90.9	95.0	89.5	93.6
European/US						
CsA/Rapa 2 mg/Pred	16.9		NA	NA	NA	NA
½ CsA/Rapa (10–20 ng/ml)/ Pred ± CsA elimination	18.5		NA	NA	NA	NA
European 1						
CsA/Aza Pred (control)		38	90	100	NA	NA
Rapa/Aza/Pred		41	98	98	NA	NA
European 2						
CsA/MMF/Pred (control)		18.4	89.5	94.7	NA	NA
Rapa/MMF/Pred		27.5	92.5	97.5	NA	NA

Abbreviations are: CsA, Cyclosporin; ½ CsA, ½ dose Cyclosporin; Aza, azathioprine; Pred, prednisolone; MMF, mycophenolate mofetil; NA, not available.
[a] Statistically significant difference vs. control

are distinct from the FKBP12 complex [54]. However, these are not well understood at the present time.

Human studies

In September 1999, Rapa received approval from the U.S. Food and Drug Administration for marketing as an agent for the prevention of acute rejection in renal transplant recipients. This was based on early results from several multicenter prospective randomized trials (RCT) that are currently ongoing. These include a United States (N = 719, 38 centers), a Global (N = 576, 34 centers), a combined European-United States (N = 247), and two European studies (N = 83 and 78, 11 and 14 centers, respectively).

Both U.S. and Global studies administered combinations of Rapa (fixed dose 2 or 5 mg/day), CsA [and prednisolone (Pred)] in an attempt to take advantage of the immunosuppressive synergy between these agents. The U.S. study gave azathioprine (Aza; 2 to 3 mg/kg) to controls, whereas the global study gave a placebo. Two-year patient and graft survival data from both Global and U.S. groups have been recently presented [Abstracts 957, 960, 962; *American Society of Transplantation and Transplant Surgeons* (AST/ASTS), Chicago, IL, USA, May 2000]. The incidence of acute rejection was clearly reduced in both trials when patients received either 2 or 5 mg/day of Rapa (Table 2). In addition, the severity of acute rejection was reduced in patients receiving Rapa at both doses, but this was only statistically significant in the Global study. The additional benefit from the higher (5 mg) dose of Rapa was small in both studies. However, an analysis of the effect of race on the efficacy of Rapa in the U.S. study demonstrated that African American patients receiving Rapa 2 mg/day had significantly higher rates of acute rejection compared with non-African Americans (30.2 vs. 13.1%). Rapa 5 mg/day produced similarly low rates of acute rejection that were less than controls in both racial groups (14.8 vs. 11.3 vs. 28.6 to 30.3%), suggesting that this dose of Rapa should be used in African American patients. Both Global and U.S. studies had comparable graft and patient survival at one year for both 2 and 5 mg/day doses with little appreciable attrition over the second year of follow-up (Table 2). Similar, if not better, results were observed from a single center (Houston, TX, USA) [55]. The same center investigated the efficacy of a Rapa (0.5 to 7 mg/kg/m^2])/CsA/Pred regimen (N = 43) versus CsA/Pred (N = 126) in patients with IgG anti-human lymphocyte antigen panel reactive antibodies (PRA) >10%. These individuals have an increased risk of acute rejection and poor graft survival. There was a statistically significant reduction in the frequency of acute rejection in those patients on Rapa (11.6 vs. 67%, $P < 0.001$), confirming the immunosuppressive efficacy of CsA/Rapa combinations in high-risk recipients (Abstract 155; *AST/ASTS Meeting*, May 2000).

The success of work combining full-dose CsA and Rapa encouraged the introduction of regimens that maintained the synergy between these agents yet minimized nephrotoxicity and side effects. Most work has focused on reducing CsA exposure, but one study has attempted steroid withdrawal. One hundred forty-nine de novo renal allograft recipients were randomized to receive Rapa or placebo with the addition of either full- or half-dose CsA (month 1 trough levels ~300 or ~200 ng/mL, respectively) and steroids [56]. The incidence of acute rejection within six months of transplantation in patients

receiving full-dose CsA was reduced from 32 to 8.5% in patients receiving Rapa (1 to 3 mg/m^2/day). Similarly low rates of acute rejection (10.7%) occurred in Caucasians treated with Rapa (1 to 5 mg/m^2/day) and reduced-dose CsA, but not in African Americans (39%), suggesting that this type of regimen is satisfactory for "low-risk" recipients but is less acceptable for those at "higher risk." Those receiving reduced-dose CsA tended to have better renal function compared with full-dose CsA recipients, but this was only statistically significant at a Rapa dose of 1 mg/m^2/day, three months after transplantation. A similar European/U.S. prospective multicenter study (N = 247) currently ongoing compares full-dose CsA and fixed-dose Rapa (2 mg/day) with reduced-dose CsA and concentration-controlled Rapa (10 to 20 ng/mL; Abstract 958; *AST/ASTS Meeting*, May 2000). The incidence of acute rejection at two months was equally low (13.5 vs. 10.9%). However, the aim of this study was to withdraw reduced-dose CsA from patients who had not rejected in the first three-months post-transplantation. This was achieved in 51 out of 77 (66%) patients, and at six months, the incidence of acute rejection in both groups was similar (Table 2) with significantly improved renal function in those who had CsA reduction/withdrawal (mean creatinine 123 vs. 147 μmol/L).

In contrast to the late elimination of CsA in low-risk patients, one pilot study (N = 6) has examined the effect of CsA exclusion in the immediate post-transplant period in allografts with a high risk of delayed graft function [57]. On the basis of this work, a further trial in 24 patients was undertaken (Abstract 571; *AST/ASTS Meeting*, May 2000). A combination of anti-CD25 monoclonal antibody (Basiliximab; Novartis, Basel, Switzerland), Rapa (5 to 10 mg to maintain 5 to 20 ng/mL trough levels), and Pred was used until serum creatinines fell below 2.5 mg/dL (166 μmol/L) when CsA was introduced. There were three episodes of acute rejection, and 23 out of 24 allografts functioned 80 days to 20 months post-transplantation with serum creatinine values below 2 mg/dL (133 μmol/L) in 80% of the patients. Regimens avoiding contributions made by CsA nephrotoxicity to delayed graft function in high-risk allografts may have significant advantages over current therapeutic options. Non–heart-beating organs with their well-documented ischemia-reperfusion injuries are obvious potential beneficiaries, but randomized controlled trials will be required to confirm this observation. CsA dose reduction (80%) with the addition of Rapa has also been performed in established delayed graft function [58]. Some, albeit impaired, renal function was salvaged presumably because CsA nephrotoxicity was reduced, while simultaneously maintaining adequate immunosuppression. However, Rapa may not be the best agent in these circumstances, as it takes several days to achieve adequate therapeutic levels, and thus, a period of underimmunosuppression can theoretically occur.

The withdrawal of steroids from CsA-based regimens has caused high rates of acute rejection in the past [59, 60]. A single-center prospective study withdrew steroids from 124 patients on CsA/Rapa combinations a mean of 437 days after transplantation (Abstract 423; *AST/ASTS Meeting*, May 2000). At two years of follow-up, only 4% had episodes of acute rejection, with 76.6% remaining off from steroids. These individuals had stable renal function and a low incidence of chronic rejection, indicating that this approach has significant potential, particularly for pediatric transplant recipients.

The two major European, prospective, randomized multicenter studies have adopted a different approach to the use of Rapa [61, 62]. Both compared Rapa triple therapy directly against CsA triple therapy in an attempt to determine whether Rapa could replace CsA as the primary immunosuppressant for renal allograft recipients. Doses of both drugs were adjusted according to trough levels (CsA, 200 to 400 ng/mL for 2 months and 100 to 200 ng/mL thereafter; Rapa, 30 ng/mL for 2 months and 15 ng/mL thereafter). The first study used Aza (2 mg/kg) and Pred, whereas the second used mycophenolate mofetil (MMF) 1 g twice daily and Pred as additional agents. At one-year follow-up, graft survival, patient survival, and incidence of acute rejection showed no statistically significant differences when CsA was compared with Rapa (Table 2). MMF combined with either CsA or Rapa produced lower rates of acute rejection than these agents and Aza. However, even patients on MMF and Rapa had a tendency to require treatment for acute rejection with bolus steroids more often than those on CsA and MMF (20 vs. 11, P = 0.068). This suggests that there may be a small price to pay for completely avoiding the nephrotoxicity of CsA. However, the clinical significance of this is debatable as renal function tended to be better in Rapa-treated patients over follow-up in both studies, although this was not statistically significant at all time points in either of the two studies.

Currently, only one uncontrolled study has combined Rapa and FK506 in human transplantation [63]. Thirty-two allograft recipients [liver (N = 23), liver/kidney (N = 2), pancreas (N = 2), or pancreas/kidney (N = 5)] received Rapa 5 mg/day (3 to 7 ng/mL), FK506 0.03 mg/day (one third of the usual dose, 6 to 12 ng/mL), and Pred with doses adjusted to trough levels. There was 94% patient survival at 230 days and only one episode of acute rejection in a noncompliant patient. Renal function was excellent, with mean serum creatinines of 93 and 112 μmol/L in hepatic and renal recipients, respectively. The combination of low-dose FK506 and Rapa provided good immunosuppression with minimal nephrotoxicity

and should be investigated further in multicenter single organ randomized controlled trials.

Rapa has also been used to treat refractory acute rejection not responding to either conventional pulsed steroid or antilymphocyte preparations [64]. Patients ($N = 21$) were given Rapa (7 mg/m^2 for 5 days, followed by 5 mg/m^2 thereafter) with no change in CsA dose (Abstract 963; *AST/ASTS Meeting*, May 2000). Ninety percent of patients had successful reversal of rejection producing an actuarial one-year graft survival of 81%. These individuals had a mean serum creatinine of 2.4 mg/dL (160 μmol/L) at one year. Refractory rejection often has a poor prognosis, with many patients requiring dialysis. These results provide reason for optimism, but larger studies are necessary to confirm these findings.

NEPHROTOXICITY OF RAPAMYCIN

Cyclosporine A and FK506 have their therapeutic benefit limited by acute and chronic nephrotoxicity. Calcineurin phosphatase inhibition is thought to be responsible for both afferent renal vasoconstriction and the release of cytokines that are responsible for these changes. Rapa has a different mechanism of action, and thus, the nephrotoxicity seen with calcineurin inhibition does not occur.

However, supratherapeutic doses of Rapa (1.5 to 10 mg/kg/day) may cause small reductions in creatinine clearance in rat native kidneys [65–67]. Work in both pigs (0.1 to 0.4 mg/kg) and rats (5 mg/kg/day) has shown that Rapa has no deleterious effects on glomerular filtration rate (GFR) or renal blood flow (RBF) and caused minimal morphological signs of toxicity [67, 68]. Rapa reduced medullary concentrating ability and increased tubular enzymuria in rat kidneys, suggesting that mild tubular injury may occur [69]. In humans, pooled data from both European trials comparing Rapa with CsA triple therapy showed a statistically significant improvement in both serum creatinine and calculated GFR at two years of follow-up in Rapa patients (mean creatinine <120 μmol/L and mean GFR >65 mL/min). This suggested that even if Rapa caused mild tubular damage, it had little clinical significance (Abstract 848; *AST/ASTS Meeting*, May 2000). However, controversy exists as to whether Rapa potentiates CsA nephrotoxicity. In a salt-depleted rat model of CsA toxicity, this combination produced a functional and morphological deterioration [70]. However, it is difficult to interpret the relevance of these findings, as high CsA trough levels (maximum 2850 ng/mL) were assessed but Rapa levels were not. A study in the Wistar-Firth rat suggested that the observed decrease in GFR could be ascribed to a Rapa-related increase in CsA concentrations, but work is necessary to characterize this further (Abstract 206; *AST/ASTS Meeting*, May 1999). In humans, Rapa (1 to 13 mg/m^2/day) was given to 30 stable renal transplant recipients taking CsA at normal therapeutic doses with no significant effects on GFR or creatinine clearance over a 14-day period [71]. However, patients in the Global/U.S. studies on Rapa tended to have higher serum creatinine levels than controls. The decreased renal function has yet to be adequately explained. Currently, only one retrospective single-center study has attempted to address this issue. CsA average concentrations (C_{AV}) and Rapa trough levels (TL; 655 samples, 96 patients) were compared with the calculated GFR at each time point (Abstract 156; *AST/ASTS Meeting*, May 2000). Increasing Rapa exposure was not associated with decreased renal function. However, as Rapa TLs increased the CsA, C_{AV} necessary to achieve optimal renal function fell. Further prospective studies are clearly required to determine whether Rapa does indeed increase CsA concentrations or whether the CsA/Rapa combination causes an independent pharmacodynamic effect.

SIDE EFFECTS OF RAPAMYCIN

Rapa is poorly tolerated by certain species. Dogs given Rapa at low doses (0.05 mg/kg/day) developed anorexia, fever, vomiting, leukocytosis and hyperamylasemia. Higher doses (0.3 to 2.0 mg/kg/day) are often fatal and autopsy findings suggest that Rapa produces a submucosal vasculitis with mucosal erosion/ulceration [31, 39, 40]. A similar but less frequent syndrome occurs in baboons [43] but is not seen in other species. In certain rat models, Rapa has been linked with myocardial necrosis, but preexisting parvovirus infection is now thought to be responsible for this observation.

Regular administration of Rapa produces a number of side effects in humans. These can occur at low daily doses (1 to 2+ mg/day) and include headaches, polyarthralgia, mild stomatitis, epistaxis, diarrhea, and skin complaints, for example, mild acne. However, myelosuppression, hyperlipidemia, and problems related to overimmunosuppression remain the major concerns.

Myelosuppression

This was first observed in mice when Rapa delayed recovery from 5-fluorouracil-induced leukopenia and thrombocytopenia in a reversible manner [72]. The underlying mechanism is unknown, but may relate to inhibition of signal transduction from hematological growth factor receptors that have sequence homology with the cytokine receptors whose action is inhibited by Rapa (for example, the IL-11 receptor that stimulates platelet production has the same gp130 β chain as the IL-2 receptor [73, 74]).

Rapa produced a dose-dependent reversible thrombocytopenia in stable renal transplant recipients within two weeks that persisted but improved over treatment. Rapa

doses of 1 to 3, 5 to 6, and 7 to 13 mg/m^2/day reduced mean platelet counts by 14, 80, and 97 cells/mm^3, respectively. A reversible leukopenia also occurred, with a mean reduction of approximately 2 cells/mm^3 for all Rapa doses [71]. The European multicenter (Aza/MMF) studies documented thrombocytopenia in 37 and 45% of Rapa patients (controls 0 and 8%, respectively, $P < 0.05$). Thirty-nine percent on Rapa/Aza/Pred had leukopenia (control 14%, $P < 0.05$), but in those receiving MMF, there was no significant difference between Rapa and controls (28 vs. 18%). CsA/Rapa combinations produced a similar incidence and severity of leukopenia/thrombocytopenia that correlated with Rapa TLs (discussed later) [55]. Patients taking Rapa/CsA recovered from a low hemoglobin after transplantation less effectively than controls.

Myelosuppression has not proved to be a significant clinical problem in the majority, but may become pathological in a small minority of patients. Rapa dose reduction or discontinuation causes initial signs of recovery usually apparent within five days. If anemia is severe, subcutaneous erythropoietin can supplement this in the short term until the hemoglobin is acceptable.

Hyperlipidemia

Both calcineurin inhibitors and steroids promote hyperlipidemia [75], glucose intolerance [76], and hypertension [77], which are well-recognized risk factors for the development of cardiovascular disease. In contrast, Rapa has little influence on blood pressure or serum glucose concentrations, and an early study in pigs reported that hyperlipidemia was mild and similar to that observed with CsA [41]. Human phase I studies were also encouraging with only higher Rapa doses (5 to 13 mg/m^2/day) causing statistically significant hypercholesterolemia and no significant effects on triglycerides [73]. However, these data only reflected a 15-day course, and longer administration has shown a different picture.

The European (Aza) study comparing Rapa and CsA triple therapy noted that Rapa caused significantly more frequent and severe hypercholesterolemia and hypertriglyceridemia than CsA (44 vs. 14% and 51 vs. 12%, respectively). Hyperlipidemia was maximal after two months [triglycerides (TGs) 5.3 vs. 2.1 mmol/L, $P < 0.001$, cholesterol (Chol) 9.2 vs. 6.4 mmol/L, $P < 0.001$] when Rapa target levels were high (30 ng/mL), but as these were reduced (15 ng/mL), this improved (6-month TG, 3.6 vs. 1.6 mmol/L, $P = 0.007$; 6-month Chol, 6.9 vs. 6.1 mmol/L, $P = 0.15$) [61]. A combined analysis of both U.S. and Global studies has shown a similar picture. Rapa produced a dose-related increase in Chol and TGs by three months that was persistent but reduced after one year of follow-up (Abstract 955; *AST/ASTS Meeting*, May 2000). The use of statins/fibrates was effective in the majority, causing significant decreases in Chol/TG levels, respectively, with few serious clinical consequences. Hypertriglyceridemia can be severe with CsA/Rapa regimens (11.7 to 42 mmol/L), particularly at higher Rapa doses, but is reversible after dose reduction or cessation of Rapa [78]. However, discontinuation of Rapa in the Global/U.S. trials was rare (0.4% Rapa 2 mg, 2.5% Rapa 5 mg). Analysis of one-year cholesterol values using The Framingham Model suggested that Rapa would cause only a small increased incidence of ischemic heart disease in renal transplant recipients (2 and 3 new cases/1000 persons/year; Rapa 2 and 5 mg, respectively). Dose reduction or elimination of CsA from Rapa patients may reduce this risk further, and results are awaited with interest.

FK506 may be less lipogenic than CsA, and thus, combination with Rapa may produce less frequent and severe hyperlipidemia. Serum Chol and TGs were elevated (Chol 5.8 mmol/L, TGs 4.6 mmol/L), but only one patient required treatment in an initial pilot study [63]. Hyperlipidemia appears less marked than with Rapa/CsA combinations, but a randomized trial to compare these regimens is necessary to confirm this preliminary data.

Overimmunosuppression

Overimmunosuppression predisposes patients to both typical/atypical infections as well as increasing the risk of neoplasia and post-transplant lymphoproliferative disease (PTLD). The European (Aza) study noted a higher incidence of herpes simplex (24 vs. 10%, $P = 0.08$) and pneumonia (17 vs. 2%, $P = 0.03$) in Rapa patients compared with controls [61]. However, when MMF replaced Aza as the secondary agent, the incidence of herpes simplex was similar in both Rapa and control patients, and although an increased incidence of pneumonia occurred, it was not statistically significant (15 vs. 5%) [62]. The multicenter trials combining CsA and Rapa reported similar significant increases in herpes simplex infections (Global) and pneumonia (United States). However, the incidence of life-threatening infections and CMV was not increased despite the immunosuppressive synergism between these drugs. The study combining FK506 and Rapa anecdotally stated that the rate and severity of bacterial/viral infection were lower than that usually seen, but no data were presented to substantiate this observation [63].

Clinical use of Rapa is still at an early stage, and its impact on PTLD is currently unknown. Kahan has followed up 250 patients treated with Rapa (3 to 48 months) and noted only two cases of PTLD (0.8%) [58], a statistic comparable to the general transplant population [79]. Multiple small bowel segments were involved in one and nuchal lymph nodes in the other. Rapa withdrawal and CsA dose reduction resulted in remission with both patients alive and well 6 and 36 months later, respectively. A two-year follow-up of 1295 patients from the Global and U.S. trials showed that only two patients

(0.7%) died as a result of neoplasia. Neither European multicenter study reported a case of neoplasia. Rapa has antiproliferative properties and was initially investigated as an antitumor agent (Abstract 141; *Cold Spring Harbour Meeting on Cell Cycle*, New York, May 1994). How this will affect the predisposition to neoplasia caused by its immunosuppressive efficacy will only become apparent over long-term follow-up.

THERAPEUTIC MONITORING OF RAPAMYCIN

Rapa, currently only available in liquid formulation, has a relatively low bioavailability (14 to 15%) with significant interindividual and intraindividual pharmacokinetic differences. It has a long half-life (approximately 63 hours), justifying both a loading dose to rapidly attain steady-state concentrations and once-daily dosing. A solid formulation (currently unlicensed) has a comparable bioavailability with similar safety and efficacy parameters (Abstracts 157, 158, and 159; *AST/ASTS Meeting*, May 2000). Conversion from liquid to solid "tablet-based" Rapa was safe and easily achievable [80].

Analysis of the U.S. and Global trials has shown that although mean Rapa levels were related to the Rapa dose, there was wide overlap in Rapa trough levels over the 2.5-fold dose range used [Rapa 2 mg, 8.06 to 8.59 ($\pm$ 4.01 to 4.03), range 2.34 to 31.30 ng/mL; Rapa 5 mg, 17.3 ($\pm$ 7.35 to 8.20), range 4.85 to 50.90 ng/mL; mean ($\pm$ SD)]. This is thought to be due to the combined action of the P450 cytochrome (CYP) 3A4 responsible for the metabolism of Rapa and the multidrug efflux pump, p-glycoprotein [81]. These are located in the liver and small bowel. Biopsies have shown that there is wide variability in the intestinal content of these proteins [82]. In addition, the activity of both is affected by coadministration of drugs such as CsA. In rats, both CsA and Rapa trough levels were increased when these were combined [83]. In humans, however, the quantitative effects of this interaction are less clear. One study in stable renal transplant recipients demonstrated that the relative timing of administration was important [84]. When CsA and Rapa were administered together, Rapa exposure was 50% higher than when they were given four hours apart. The timing of administration did not affect CsA levels. Further studies are required to characterize this relationship more extensively.

A good correlation exists between Rapa trough levels and the more accurate assessment of drug exposure, area under the concentration curve [85]. Therefore, current recommendations for monitoring Rapa are based on trough levels. Regression analysis of data from the U.S. and Global trials has suggested that in order to minimize the risk of acute rejection, Rapa levels should not fall below approximately 3.5 ng/mL during concomitant administration of full-dose CsA and steroids [86]. However, most would agree that 5 ng/mL is a safer estimate of the lower end of Rapa's "therapeutic window" [87]. This may not apply in high-risk patients, for example, African Americans who may need to have levels nearer 15 ng/mL to maintain a low incidence of acute rejection. The upper end of the therapeutic window aims to minimize toxicity. The distribution frequencies for platelet count, Chol and TGs by Rapa concentration ranges suggested levels of $>$15 ng/mL were associated with the greatest derangement. A study from Houston documented a linear correlation between these side effects and Rapa trough levels [55]. Rapa levels $>$15 ng/mL caused a 25% fall in platelet count and a 50% increase in TGs compared with Rapa levels $<$5 ng/mL, supporting the use of 15 ng/mL as a sensible upper limit.

The successful reduction in acute rejection associated with fixed Rapa doses of 2 and 5 mg/day in the U.S. and Global trials suggests that while desirable, routine monitoring of Rapa levels in such regimens is not essential, particularly at doses of 2 mg/day. However, monitoring is probably necessary to ensure safe, effective concentrations in (*1*) the early period post-transplantation to confirm that levels are not at the extremes of the expected range [two determinations within the first two weeks are advised due to the intraindividual variability in Rapa pharmacokinetics; if a dose change is necessary, a further assessment should be made when a new steady state has been attained (5 to 7 days after dose change)]; (*2*) after the introduction/discontinuation of CYP 3 A4/P-glycoprotein inhibitors/inducers to ensure safe, effective concentrations; (*3*) patients in whom CsA dose or the relative timing of Rapa/CsA doses is altered significantly; (*4*) there are signs of toxicity, for example, hyperlipidemia, or a change in clinical condition, for example, development of liver disease; and (*5*) in patients in whom noncompliance is suspected. Closer monitoring of Rapa levels is recommended in patients at high risk of rejection, children, and those with hepatic impairment.

The problem currently facing clinicians using Rapa is that most centers do not have a validated method to analyze Rapa levels, and therefore, samples are sent to reference laboratories. This can introduce significant delays, making patient management cumbersome. Methods with a faster turnaround need to be developed or centers will have to introduce techniques locally. There are currently four published techniques to monitor Rapa levels. The gold standard is high-performance liquid chromatography with mass spectrometric (HPLC-MS) detection, but this is expensive, not widely available, and requires significant operator expertise [88]. HPLC with ultraviolet detection is available in many laboratories, and several methods have shown a good correlation with HPLC-MS, supporting its use in a clinical setting [88]. However, it is quite time consuming, taking between 12 and 14 hours to process 24 samples. Therefore, the use

of microparticle enzyme immunoassays (MEIAs), which can analyze 24 samples within two hours thus facilitating a rapid turnaround, has been advocated [89]. Although this is able to measure Rapa levels within the therapeutic range, it is not specific for Rapa alone, detecting Rapa metabolites that have little biological activity. Therefore, when compared with HPLC, MEIA produces a positive bias of approximately 20%. The practical significance of this remains to be assessed. Immunophilin binding assays (IBAs), although showing weak Rapa metabolite cross-reactivity, correlate with HPLC values, are easier to perform, and have the potential to be automated [90]. Since an automated system will ultimately be required as Rapa use becomes widespread, this may offer a good option for the future. However, the field is evolving rapidly and techniques such as monitoring Rapa levels by p70 S6 kinase inhibition are under development [91].

CHRONIC REJECTION

The histology of chronic allograft rejection (CR) is dominated by the accumulation of extracellular matrix. This is characterized by intimal hyperplasia, a concentric vasculopathy, and interstitial fibrosis in addition to organ-specific changes that may reflect the effects of ischemia secondary to this initial vascular involvement [92]. It is thought that early endothelial damage initiates a cascade of growth factor production, for example, transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) [93, 94], resulting in recruitment and proliferation of inflammatory cells, smooth muscle cells, and fibroblasts with an ensuing up-regulation of extracellular matrix deposition and scarring [95, 96]. A number of lines of evidence suggest that Rapa may theoretically influence the course of CR.

(1) Rapa inhibits growth factor-mediated proliferation of cells involved in the pathogenesis of CR in vitro. This antiproliferative effect is seen in nonstimulated as well as basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), and PDGF-driven vascular smooth muscle cells [97–99], and was greater with Rapa than MMF or FK506 [100]. Rapa exerted similar effects on bFGF-induced bovine aortic and human umbilical vein endothelial cells as well as PDGF/bFGF-stimulated rat cardiac and human lung fibroblasts [100–103]. These antiproliferative properties were antagonized by combination with FK506 in molar excess, suggesting that the formation of the Rapa/FKBP12 complex is an important underlying mechanism [104]. However, Rapa does not uniformly inhibit cell proliferation. Some hematopoietic and epidermoid cell lines are unaffected by its administration [105, 106]. In addition, there is currently little evidence that Rapa inhibits cell proliferation stimulated by arguably one of the most important cytokines in the development of CR: TGF-β.

(2) Intimal hyperplasia (often associated with CR) is a stereotyped response of the endothelium to both immune and nonimmune injury. Rapa treatment reduced the degree of intimal thickening and associated local cytokine expression at doses of 1.5 mg/kg/day after mechanical injury (angioplasty balloon catheter). However, it was initially thought to be ineffective at this dose in a femoral allograft model of immune injury [107]. These findings were criticized because the intimal hyperplasia was analyzed near the suture line of femoral allografts and thus may have been excessive. On re-evaluation of the central part of femoral allografts, intimal hyperplasia was reduced by 1.5 mg/kg/day Rapa, as seen in the mechanical model [108]. Rapa was most effective at higher doses (6 mg/kg/day), but lower dose Rapa (1.5 mg/kg/day) in combination with MMF was also highly effective after balloon catheter damage. Administration of Rapa and MMF for 14 days after injury inhibited intimal hyperplasia at day 14, but by day 40, intimal hyperplasia had returned and was similar to untreated controls. However, if administered 3 days before and 14 days after insult, there was little intimal hyperplasia observed at day 40 [109], presumably as sufficient concentrations were present to dampen the initial cytokine cascade immediately after injury, generating a smaller intimal response that resolved with few sequelae. These studies show that the vascular response to both immune and nonimmune injury is inhibited by Rapa, but the interplay between the antiproliferative and immunosuppressive properties that contribute to this is unclear. On one hand, the balloon injury model lends support to an antiproliferative theory. On the other, Rapa (3 mg/kg/3 times/week) prevented both acute rejection and arteriosclerotic changes in rat aortic allografts [110]. The positive correlation noted between acute rejection and graft arteriosclerosis suggested that immunosuppressive effects were also important in the attenuation of vascular injury.

At the molecular level, rat aortic allografts treated with Rapa (0.5 mg/kg/day) had little intimal hyperplasia but significant inducible nitric oxide synthase (iNOS) expression at 30 days. In contrast, CsA treatment was associated with marked intimal hyperplasia but no detectable iNOS expression [111]. Nitric oxide (NO) inhibited the proliferation of vascular smooth muscle cells in both balloon-injured and aortic allograft models, and also prevented the development of arteriosclerosis [112, 113]. Thus, one possible explanation for Rapa's actions is that iNOS expression is maintained, causing high local NO levels that limit vascular smooth muscle responses to injury. Future work combining Rapa with selective iNOS inhibitors will delineate this process further.

Rapa also reduced the severity of established intimal hyperplasia in rat femoral artery allografts when given 14, 21, and even 30 days after the onset of alloimmune injury [108]. However, larger Rapa doses were required

(3 to 6 mg/kg/day), and intimal hyperplasia was most successfully reduced when Rapa was added at an early stage, that is, when intimal hyperplasia was relatively immature. A similarly large dose of Rapa (6 mg/kg/day) prevented the development of obliterative bronchiolitis (compare CR) in rat tracheal allografts more effectively than CsA or MMF. No luminal fibrosis or loss of respiratory mucosa was demonstrated [114], suggesting that Rapa may have extravascular as well as intravascular effects in certain models.

(3) The effect of Rapa on CR in whole organs has been investigated in rat cardiac allografts where it is manifested as graft vessel disease (GVD). Meiser, Billingham, and Morris were the first to observe that Rapa inhibited GVD in such circumstances [115]. Further work has shown that inhibition of transplant vasculopathy and graft vessel luminal obstruction secondary to myointimal changes (100 days postoperatively) was most effective when Rapa was given at high doses (5 mg/kg/day for 14 days followed by 2.5 mg/kg/day thereafter) [116]. Lower Rapa doses (0.5 and 2 mg/kg/day) were less effective but did still produce a significant reduction in vasculopathy (control 59 ± 7% vs. Rapa 0.5 mg/kg/day 25 ± 15% vs. Rapa 2 mg/kg/day 22 ± 11%). The addition of Rapa (0.5 mg/kg/day) to low-dose CsA (1.5 mg/kg/day) for 14 days reduced lymphocytic infiltration but did not cause an additional reduction in vasculopathy. The immunosuppressive synergism between these agents appeared to have little beneficial impact on GVD at the low doses used over this short time period. However, the combination of CsA (15 mg/kg/day) and Rapa (1 mg/kg/alternate days) for 12 weeks after rat cardiac allografting reduced the frequency and severity of both acute rejection and GVD compared with CsA alone [117]. Rapa/CsA allografts showed sparse infiltration of inflammatory cells, little expression of adhesion molecules, no expression of IFN-γ/TNF-α and little GVD. These findings suggest that the synergism between Rapa and CsA plays an important role in the inhibition of chronic rejection, contradicting earlier work. Wasowska et al confirmed these findings [118] and also investigated the expression of monocyte associated chemokines involved in the development of arteriosclerosis [119]. The expression of IL-12, MCP-1, and RANTES were significantly decreased by CsA/Rapa therapy, providing further evidence that the synergism between these drugs may be beneficial in GVD.

Rats with established cardiac GVD received Rapa 3 mg/kg/day over a 30-day period two months after transplantation in order to determine whether this process was reversible [120]. Rapa compared with an equipotent dose of CsA led to a significant reduction in GVD, although the overall level of graft inflammation and perivascular infiltrate was similar, implying analogous levels of immunosuppression. Presumably Rapa had an antiproliferative effect causing the reduction in GVD. As with models of vascular injury, both the antiproliferative and immunosuppressive properties of Rapa seem important in the prevention of CR in cardiac allografts. However, further work using renal allografts and some of the better characterized growth factors linked with CR, for example, TGF-β, PDGF, bFGF, is required to determine the magnitude of these benefits for renal transplant recipients.

(4) Underimmunosuppression and severe, recurrent, or late episodes of acute rejection are risk factors for CR. Rapa has been shown to be a powerful and effective immunosuppressant, particularly in combination with CsA, where both rates and severity of acute rejection were lower than controls. This may have a significant impact on the incidence of CR in the future, but only long-term follow-up will confirm its efficacy.

(5) CsA causes chronic nephrotoxicity, generating both fibrotic changes and an up-regulation of profibrotic cytokines that can be indistinguishable from those of CR. Rapa is non-nephrotoxic and has the potential to allow early dose reduction or elimination of CsA, limiting the adverse effects of this drug on the development of CR. Such studies have recently been instituted and have been discussed earlier in this article. However, it will be several years before meaningful data with respect to CR become available. Studies using surrogate markers, for example, RT-PCR of profibrotic genes and computerized histomorphometry of fibrosis of protocol biopsies, may allow a prediction of Rapa's impact on CR in the interim [121].

Patients with established chronic allograft nephropathy who have 40 to 50% CsA dose reductions with the addition of Aza or MMF can have significant improvements in renal function [122, 123]. A similar CsA reduction with the addition of Rapa may also be beneficial. A small prospective randomized trial ($N = 31$) compared a 40% CsA dose reduction alone to the same CsA dose reduction with the addition of Rapa 2 mg/day in patients with chronic allograft nephropathy (Abstract 432; *AST/ASTS Meeting*, May 2000). Over a six-month period, renal function was not improved by the addition of Rapa. A study including larger numbers of patients with a longer follow-up is required to provide more definitive evidence in this respect.

There are two problems with the hypothesis that Rapa may prevent/limit CR in humans. First, although the pathogenesis of CR is multifactorial, hyperlipidemia may play a role [124]. Oxidized low-density lipoprotein (LDL) is toxic to endothelial cells and induces endothelial and smooth muscle cell proliferation as well as macrophage differentiation into foam cells in vitro. Animals with fatty acid deficiencies are protected from CR, but the addition of large amounts of dietary cholesterol accelerates the development of proliferative vascular lesions [125]. In

humans, there are several studies documenting an association between hyperlipidemia and CR [124]. Rapa is known to cause hyperlipidemia in a dose-dependent manner. The therapeutic benefits of Rapa with respect to CR may be reduced by this tendency. Hyperlipidemia can usually be corrected by appropriate medication. However, starting patients on one drug to counteract the effects of another raises the issue of poly-pharmacy, which can produce significant side effects as well as incurring substantial costs that may or may not be justified by improved long-term outcome.

Arguably, a more important issue is whether the relatively low doses of Rapa given to humans (0.2 to 0.3 mg/kg/day Rapa alone and 0.01 to 0.17 mg/kg/day Rapa/CsA combinations) are large enough to effectively inhibit CR. In rat models, the inhibition of intimal hyperplasia occurs at 1.5 mg/kg/day and increases significantly as Rapa doses are elevated. If such doses were administered to humans, side effects and overimmunosuppression would be poorly tolerated.

In summary, it is plausible that Rapa may limit the progression of CR, although long-term results from the current multicenter studies will ultimately show whether concerns re-hyperlipidemia and Rapa dose are important. The most significant advantages will probably be seen in recipients of de novo allografts given higher doses of Rapa prior to induction, but patients with established CR may also benefit.

CONCLUSION

This review has demonstrated that there is good in vitro and in vivo evidence supporting the use of Rapa in renal transplant recipients. Rapa reduces the incidence of acute rejection in de novo transplants when given alone, but seems to be most effective in combination with CsA. Regimens utilizing CsA reduction or elimination are currently under investigation and should reduce nephrotoxicity and side effects. Monitoring of Rapa blood levels will be necessary in some patients, and techniques are being developed to make this more widely available. Furthermore, several lines of evidence suggest that Rapa may retard the development and progression of CR, although hyperlipidemia and dose limitations may reduce its potential therapeutic effects.

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APPENDIX

Abbreviations used in this article are: Aza, azathioprine; bFGF, basic fibroblast growth factor; C_{AV}, CsA average concentrations; Chol, cholesterol; CR, chronic allograft rejection; CsA, cyclosporine A; CYP 3A4, cytochrome 3A4; FK506, tacrolimus; FKBP12, FK binding protein 12; GFR, glomerular filtration rate; GVD, graft vessel disease; HPLC-MS, high-performance liquid chromatography-mass spectrometry; IBA, immunophilin binding assay; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL, interleukin; iNOS, inducible nitric oxide synthase; MEIA, microparticle enzyme immunoassays; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; NO, nitric oxide; PDGF, platelet-derived growth factor; PRA, panel reactive antibodies; Pred, prednisolone; PTLD, post-transplant lymphoproliferative disease; Rapa, Rapamycin; RBF, renal blood flow; RCT, randomized controlled trials; RT-PCR, reverse transcription-polymerase chain reaction; TGs, triglycerides; TGF-β, transforming growth factor-β; TL, trough levels; TNF-α, tumor necrosis factor-α; TOR, target of rapamycin.

REFERENCES

1. Vezina C, Kudelski A, Sehgal SN: Rapamycin, a new antifungal antibiotic. Taxonomy of the producing Streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* 28:721–726, 1975
2. Sehgal SN, Baker H, Vezina C: Rapamycin, a new antifungal antibiotic. Fermentation, isolation and characterization. *J Antibiot (Tokyo)* 28:727–732, 1975
3. Manez R, Jain A, Marino IR, *et al*: Comparative evaluation of tacrolimus and cyclosporin as immunosuppressive agents. *Transplant Rev* 9:63, 1995
4. Sehgal SN: Rapamune (Sirolimus, Rapamycin): An overview and mechanism of action. *Ther Drug Monit* 17:660–665, 1995
5. Morris RE: New small molecule immunosuppressants for transplantation: Review of essential concepts. *J Heart Lung Transplant* 12(Suppl):S275–S286, 1993
6. Cardenas ME, Lorenz M, Hemenway C, Heitmen J: Yeast as model T cells. *Drug Des Discov* 2:103–126, 1994
7. Chiu MI, Katz H, Berlin V: RAPT1, a mammalian homologue of yeast TOR interacts with the FKBP12-Rapamycin complex. *Proc Natl Acad Sci USA* 91:12574–12578, 1994
8. Chen Y, Chen H, Rhoad AO, *et al*: A putative Sirolimus (Rapamycin) effector protein. *Biochem Biophys Res Commun* 203:1–7, 1994
9. Brown EJ, Albers MW, Shin TB, *et al*: Purification and molecular cloning of FRAP a direct target of G1 arrest complex FKBP12-Rapamycin. *Nature* 369:756–761, 1994
10. Sabers CJ, Martin MM, Brunn GJ, *et al*: Isolation of protein target of FKBP12-Rapamycin complex in mammalian cells. *J Biol Chem* 270:815–822, 1994
11. Sonenberg N, Gingras AC: The mRNA 5′ cap-binding protein eIF-4E and control of cell growth. *Curr Opin Cell Biol* 10:268–275, 1998
12. Beretta L, Gingras AC, Svitkin YV, *et al*: Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J* 15:658–664, 1996
13. Sabatini DM, Pierchala BA, Barrow RK, *et al*: The Rapamycin and FKBP12 target (RAFT) displays phospatidylinositol 4-kinase activity. *J Biol Chem* 270:20875–20878, 1995
14. Jeffries HBJ, Fumagalli S, Dennis PB, *et al*: Rapamycin suppresses 5′TOP mRNA translation through inhibition of $p70^{s6k}$. *EMBO J* 12:3693–3704, 1997
15. Chung J, Kuo CJ, Crabtree GR, *et al*: Rapamycin-FKBP12 specifically blocks growth-dependent activation of and signalling by the 70kd S6 protein kinases. *Cell* 69:1227–1236, 1992
16. Terada N, Lucas JJ, Szepesi A, *et al*: Rapamycin blocks cell cycle progression of activated T cells prior to events characteristic of the middle to late G1 phase of the cell cycle. *J Cell Physiol* 268:22825–22829, 1993
17. Sehgal SN: Rapamune: Mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem* 31:335–340, 1998
18. Bertagnolli MM, Yang L, Herrmann SH, *et al*: Evidence that Rapamycin inhibits interleukin-12 induced proliferation of activated T lymphocytes. *Transplantation* 58:1091–1096, 1994
19. Sehgal SN, Bansbach CC: An in vitro immunological profile of Rapamycin. *Ann NY Acad Sci* 685:58–67, 1993
20. Sehgal SN, Molnar-Kimber K, Ocain T, *et al*: Rapamycin: A novel immunosuppressive macrolide. *Med Res Rev* 14:1–22, 1994

21. Aaguaard-Tillery KM, Jelinek D: Inhibition of human B lymphocyte cell cycle progression and differentiation by Rapamycin. *Cell Immunol* 152:493–507, 1994
22. Kim HS, Raskova J, Degiannis D, *et al*: Effects of cyclosporin and Rapamycin on immunoglobulin production by preactivated human B cells. *Clin Exp Immunol* 96:508–512, 1994
23. Dumont FJ, Kastner CA: Transforming growth factor beta 1 inhibits interleukin-1 induced but enhances ionomycin induced interferon-γ production in a T cell lymphoma: Comparison with the effects of Rapamycin. *J Cell Physiol* 160:141–153, 1994
24. Zheng XX, Strom TB, Steele AW: Quantitative comparison of Rapamycin and cyclosporin, effects on cytokine gene expression studied by reverse transcriptase-competitive polymerase chain reaction. *Transplantation* 58:87–92, 1994
25. Kahan BD, Gibbons S, Chou TC: Synergistic interactions of cyclosporine and Rapamycin to inhibit immune performances of normal human peripheral blood leukocytes in vitro. *Transplantation* 51:232–239, 1991
26. Vathsala A, Goto S, Yoshimara N, *et al*: Immunosuppressive antagonism of FK506 and Cyclosporin at low drug doses. *Transplantation* 52:121–128, 1991
27. Dumont FJ, Melino MR, Staruch MJ, *et al*: The immunosuppressive macrolides FK506 and Rapamycin act as reciprocal antagonists in murine T cells. *J Immunol* 144:1418, 1990
28. Calne R, Collier D, Lim S, *et al*: Rapamycin for immunosuppression in organ allografting. *Lancet* 2:227, 1989
29. Morris RE, Meiser BM: Identification of a new pharmacological action for a old compound. *Med Sci Res* 17:609–610, 1989
30. Eng CP, Gullo-Brown JG, Chang JY, *et al*: Inhibition of skin graft rejection in mice by Rapamycin: A novel immunosuppressive macrolide. *Transplant Proc* 23(1 Pt 1):868–869, 1991
31. Ochiai T, Gunji Y, Nagata M, *et al*: Effects of Rapamycin in experimental organ allografting. *Transplantation* 56:15–19, 1993
32. Schmidbauer G, Hancock WW, Wasowska B, *et al*: Rapamycin abrogates accelerated rejection in sensitized rats by selectively suppressing intragraft cell activation, adhesion binding properties and modulating serum alloantibody responses. *Transplant Proc* 27:427–429, 1995
33. Stepkowski SM, Kahan BD: Rapamycin and cyclosporin synergistically prolong heart and kidney allograft survival. *Transplant Proc* 23:3262–3264, 1991
34. Stepkowski SM, Chen H, Daloze P, *et al*: Rapamycin, a potent immunosuppressive drug for vascularized heart, kidney and small bowel transplantation in the rat. *Transplantation* 51:22, 1991
35. Vu MD, Qi S, Xu D, *et al*: Synergistic effects of Mycophenolate mofetil and Sirolimus in prevention of acute heart, pancreas and kidney allograft rejection and in reversal of ongoing heart allograft rejection in the rat. *Transplantation* 66:1575–1580, 1998
36. Chen H, Wu J, Xu D, *et al*: The effect of Rapamycin on orthoptic small bowel transplantation in the rat. *Transplant Proc* 24:1157, 1992
37. Kahan BD, Chang JY, Sehgal SN: Preclinical evaluation of a new potent immunosuppressive agent, Rapamycin. *Transplantation* 52:185–191, 1991
38. Fryer J, Yatscoff RW, Pascoe EA, *et al*: The relationship of blood concentrations of Rapamycin and cyclosporin to suppression of allograft rejection in a rabbit heterotopic heart allograft model. *Transplantation* 55:340–345, 1993
39. Knight R, Ferraresso M, Serino F, *et al*: Low dose Rapamycin potentiates the effects of subtherapeutic doses of cyclosporin to prolong renal allograft survival in the mongrel canine model. *Transplantation* 55:947–949, 1993
40. St J Collier D, Calne R, Thiru S, *et al*: Rapamycin in experimental renal allografts in dogs and pigs. *Transplant Proc* 22:1674–1675, 1990
41. Almond PS, Moss A, Nakhleh R, *et al*: Rapamycin: Immunosuppression, hyporesponsiveness and side effects in a porcine renal allograft model. *Transplantation* 56:275–281, 1993
42. Granger DK, Cromwell JW, Chen S, *et al*: Prolongation of renal allograft survival in a large animal model by oral Rapamycin monotherapy. *Transplantation* 59:183–186, 1995
43. St J Collier D, Calne RY, Pollard SG, *et al*: Rapamycin in experimental renal allografts in primates. *Transplant Proc* 23:2246–2247, 1991
44. Chen HF, Luo H, Daloze P, *et al*: Long term in vivo effects of Rapamycin on humoral and cellular immune responses in the rat. *Immunobiology* 188:303–315, 1993
44. Chen HF, Wu JP, Luo HY, *et al*: Reversal of ongoing heart, kidney, and pancreas rejection and suppression of accelerated heart allograft rejection in the rat by Rapamycin. *Transplantation* 56:661–666, 1993
45. Wang M, Stepkowski SM, Ferraresso M, *et al*: Evidence that Rapamycin rescue therapy delays rejection of major and minor histocompatible heart allografts in rats. *Transplantation* 54:704–709, 1992
46. Longoria JL, Roberts RF, Marboe CC, *et al*: Sirolimus potentiates cyclosporin in prevention of acute lung rejection. *J Thorac Cardiovasc Surg* 117:715–718, 1999
47. Qi S, Xu D, Peng J, *et al*: Synergistic effect of rapamycin and cyclosporin in prevention of acute kidney allograft rejection in the mouse. *Microsurgrery* 19:344–347, 1999
48. Stepkowski SM, Tian L, Napoli KL, *et al*: Synergistic mechanisms by which sirolimus and cyclosporin inhibit rat heart and kidney allograft rejection. *Clin Exp Immunol* 108:63–68, 1997
49. Morris RE, Meiser BM, Wu J, *et al*: Use of rapamycin for the suppression of alloimmune reactions in vivo: Schedule dependence, tolerance induction, synergy with cyclosporin and FK506 and effect on host-versus-graft and graft-versus-host reactions. *Transplant Proc* 23(1 Pt 1):521–524, 1991
50. Vu MD, Qi S, Dasheng X, *et al*: Tacrolimus and Sirolimus in combination are not antagonistic but produce extended graft survival in cardiac transplantation in the rat. *Transplantation* 64:1853–1856, 1997
51. Chen H, Qi S, Xu D, *et al*: FK506 and Rapamycinin combination are not antagonistic but produce extended small bowel graft survival in the mouse. *Transplant Proc* 30:1039–1041, 1998
52. De Paulis A, Cirillo R, Ciccarelli A, *et al*: FK506 a potent novel inhibitor of the release of proinflammatory mediators from human FceRI+ cells. *J Immunol* 146:2374, 1991
53. Sigal NH, Dumont FJ: Cyclosporin A, FK506 and Rapamycin: Pharmacological probes of lymphocyte signal transduction. *Annu Rev Immunol* 10:519, 1992
54. Bierer BE, Somers PK, Wandless TJ, *et al*: Probing immunosuppressant action with nonnatural immunophilin ligand. *Science* 250:56, 1990
55. Kahan B, Podbielski J, Kimberley L, *et al*: Immunosuppressive effects and safety of a Sirolimus/cyclosporin combination regimen for renal transplantation. *Transplantation* 66:1040–1046, 1998
56. Kahan BD, Julian BA, Pescovitz MD, *et al*: Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in Caucasian recipients of mismatched primary renal allografts: A phase II trial. *Transplantation* 68:1526–1532, 1999
57. Hong JC, Kahan BD: Use of anti-CD25 monoclonal antibody in combination with Rapamycin to eliminate cyclosporin treatment during the induction phase of immunosuppression. *Transplantation* 68:701–704, 1999
58. Kahan BD: Rapamycin: Personal algorithms for use based on 250 treated renal allograft recipients. *Transplant Proc* 30:2185–2188, 1998
59. Ahsan N, Hricik D, Matas A, *et al*: Prednisolone withdrawal in kidney transplant recipients on cyclosporin and mycophenolate mofetil: A prospective randomised study. *Transplantation* 68:1865–1874, 1999
60. Schulak JA, Mayes JT, Moritz CE, *et al*: A prospective randomised trial of prednisolone versus no prednisolone maintenance therapy in cyclosporin and azathioprine treated renal transplant patients. *Transplantation* 49:327–332, 1990
61. Groth CG, Backman L, Morales J, *et al*: Sirolimus (Rapamycin) based therapy in human renal transplantation. *Transplantation* 67:1036–1042, 1999
62. Kreis H, Cisterne JM, Land W, *et al*: Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 69: 1252–1260, 2000

63. McAlister VC, Gao Z, Peltekian K, *et al*: Sirolimus-tacrolimus combination immunosuppression. *Lancet* 355:376–377, 2000
64. Slaton JW, Kahan BD: Case report: Sirolimus rescue therapy for refractory renal allograft rejection. *Transplantation* 61:977–979, 1996
65. Whiting PH, Woo J, Adam BJ, *et al*: Toxicity of Rapamycin: A comparative and combination study with cyclosporin at immunotherapeutic dosage in the rat. *Transplantation* 52:203–208, 1991
66. DiJoseph JF, Sharma RN, Chang JY: The effect of Rapamycin on kidney function in the Sprague-Dawley rat. *Transplantation* 53:507–513, 1992
67. Ryffel B, Weber E, Mihatsch MJ: Nephrotoxicity of immunosuppressants in rats: Comparison of macrolides with cyclosporin. *Exp Nephrol* 2:324–333, 1994
68. Golbaekdal K, Nielson CB, Djurhuus JC, *et al*: Effects of Rapamycin on renal haemodynamics, water and sodium excretion and plasma levels of angiotensin II, aldosterone, atrial natriuretic peptide, and vasopressin in pigs. *Transplantation* 58:1153–1157, 1994
69. Andoh TF, Burbmann EA, Fransechini N, *et al*: Comparison of acute rapamycin nephrotoxicity with cyclosporin and FK506. *Kidney Int* 50:1110–1117, 1996
70. Andoh TF, Lindsley J, Franceschini N, *et al*: Synergistic effects of cyclosporin and rapamycin in a chronic nephrotoxicity model. *Transplantation* 62:311–316, 1996
71. Murgia MG, Jordan S, Kahan BD: The side effect profile of Sirolimus: A phase I study in quiescent cyclosporin-prednisolone treated renal transplant patients. *Kidney Int* 49:209–216, 1996
72. Quesniaux VFJ, Wehrli S, Steiner C, *et al*: The immunosuppressant Rapamycin blocks in vitro responses to haemopoetic cytokines and inhibits recovering but not steady state haematopoiesis in vivo. *Blood* 84:1543–1552, 1994
73. Du XX, Williams DA: Interleukin-11: A multifunctional growth factor derived from the haemopoetic microenvironment. *Blood* 83:2023–2030, 1994
74. Paul SR, Bennett F, Calvetti JA, *et al*: Molecular cloning of a cDNA encoding interleukin-11, a stromal cell derived haematopoietic and lymphopoietic cytokine. *Proc Nat Acad Sci USA* 87:7512–7516, 1990
75. Satterthwaite R, Aswad S, Sunga V, *et al*: Incidence of new onset hypercholesterolaemia in renal transplant patients treated with FK506 and cyclosporin. *Transplantation* 65:446–449, 1998
76. Cavaille MV, Elashoff MR: A comparison of tacrolimus and cyclosporin for immunosuppression after cadaveric renal transplantation. *Transplantation* 65:142–145, 1998
77. Copley JB, Staffield C, Lindberg J, *et al*: Cyclosporin to Tacrolimus: Effect on hypertension and lipid profiles in renal allografts. *Transplant Proc* 30:1254–1256, 1998
78. Brattstrom C, Wilczek H, Tyden G, *et al*: Hyperlipidaemia in renal transplant recipients treated with Sirolimus (Rapamycin). *Transplantation* 65:1272–1274, 1998
79. Bouberudin S, Hiesse C, Goupy C, *et al*: Incidence and consequences of post transplant lymphoproliferative disease. *J Nephrol* 10:136–145, 1997
80. Kelly PA, Napoli K, Kahan BD: Conversion from liquid to solid rapamycin formulations in stable renal allograft transplant recipients. *Biopharm Drug Dispos* 20:249–253, 1999
81. Zimmerman JJ, Kahan BD: Pharmakokinetics of Sirolimus in stable renal transplant patients after multiple oral dose administration. *J Clin Pharmacol* 37:405–415, 1997
82. Wacher VJ, Wu CY, Benet LZ: overlapping substrate specificities and tissue distribution of cytochrome P450, 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* 13:129–134, 1995
83. Napoli KL, Wang ME, Stepkowski SM, *et al*: Relative tissue distributions of cyclosporin and Sirolimus after concomitant per-oral administration to the rat. Evidence for pharmacokinetic interactions. *Ther Drug Monit* 20:123–133, 1997
84. Kaplan B, Meier-Kriesche HU, Napoli KL, *et al*: The effects of relative timing of Sirolimus and cyclosporin micro-emulsion formulation coadministration on the pharmacokinetics of each agent. *Clin Pharmacol Ther* 63:49–53, 1998
85. Zimmerman JJ, Kahan BD: Pharmacokinetics of Sirolimus in stable renal transplant recipients after multiple oral dose administration. *J Clin Pharmacol* 37:405–415, 1997
86. MacDonald A, Scarola J, Burke JT, *et al*: Clinical pharmacokinetics and therapeutic drug monitoring of Sirolimus. *Clin Ther* 22:B101–121, 2000
87. Meier-Kriesche H, Kaplan B: Toxicity and efficacy of Sirolimus: Relationship to whole blood concentrations. *Clin Ther* 22:B93–100, 2000
88. Napoli KLA: Practical guide to the analysis of Sirolimus using high performance liquid chromatography with ultraviolet detection. *Clin Ther* 22:B14–24, 2000
89. Jones K, Saadat-Lajevardi S, Lee T, *et al*: An immunoassay for the measurement of Sirolimus. *Clin Ther* 22:B49–61, 2000
90. Davis DL, Soldin SJ: An immunophilin-binding assay for Sirolimus. *Clin Ther* 22:B62–70, 2000
91. Yatscoff RW, Aspeslet LJ, Gallant HL: Pharmacodynamic monitoring of immunosuppressive drugs. *Clin Chem* 44:428–432, 1998
92. Hayry P, Aavik E, Savolainen H: Mechanisms of chronic rejection. *Transplant Proc* 31:5S–8S, 1999
93. Reidy MA: Factors controlling smooth muscle cell proliferation. *Arch Pathol Lab Med* 116:1276–1280, 1992
94. Sporn MB: TGFβ problems and prospects. *Cell Regul* 12:875–882, 1990
95. Kasiske BL, Kalil RS, Lee HS, *et al*: Histopathological findings associated with a chronic decline in renal allograft function. *Kidney Int* 40:514–524, 1991
96. Painter TA: Myointimal hyperplasia: Pathogenesis and implications. *Artif Organs* 15:42–55, 1991
97. Marx SO, Jayaraman T, Go LO, *et al*: Rapamycin inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. *Circ Res* 76:412–417, 1995
98. Gregory CR, Pratt RE, Huie P, *et al*: Effect of treatment with cyclosporin, FK506, Rapamycin, MMF or deoxyspergualin on vascular smooth muscle proliferation in vitro and in vivo. *Transplant Proc* 25:770–771, 1993
99. Obata T, Kashiwagi A, Maegawa H, *et al*: Insulin signalling and its regulation of system A amino acid uptake in cultured rat vascular smooth muscle cells. *Circ Res* 79:1167–1176, 1996
100. Mohasci PJ, Tuller D, Hullinger B, *et al*: Different inhibitory effects of immunosuppressive drugs on human and rat aortic smooth muscle and endothelial cell proliferation stimulated by platelet derived growth factor or endothelial cell growth factor. *J Heart Lung Transplant* 6:484–492, 1997
101. Akselband Y, Harding MW, Nelson PA: Rapamycin inhibits spontaneous and bFGF driven proliferation of endothelial cells and fibroblasts. *Transplant Proc* 23:2833–2836, 1991
102. Simm A, Nestler M, Hoppe V: PDGF-AA, a potent mitogen for cardiac fibroblasts from adult rats. *J Mol Cell Cardiol* 29:357–368, 1997
103. Nair R, Huang X, Shorthouse R, *et al*: Antiproliferative effect of Rapamycin on growth factor stimulated human adult lung fibroblasts in vitro may explain its superior efficacy for prevention and treatment of allograft obliterative airway disease in vivo. *Transplant Proc* 29:614–615, 1997
104. Cao W, Mohasci P, Shorthouse R, *et al*: Effects of Rapamycin on growth factor stimulated vascular smooth muscle cell DNA synthesis. *Transplantation* 59:390–395, 1995
105. Hultsch T, Martin R, Hohman RJ: The effect of the immunophilin ligands Rapamycin and FK506 on proliferation of mast cells and other haematopoetic cell lines. *Mol Biol Cell* 3:981–987, 1992
106. Zeevi A, Woan GZ, Yao R, *et al*: Comparative in vitro studies on the immunosuppressive activities of MMF, Bredinin, FK506, cyclosporin and Rapamycin. *Transplant Proc* 23:2928–2930, 1991
107. Gregory C, Huie P, Billingham ME, *et al*: Rapamycin inhibits arterial intimal thickening caused by both alloimmune and mechanical injury. *Transplantation* 55:1409–1418, 1993
108. Morris RE, Cao W, Huang X, *et al*: Rapamycin inhibits vascular smooth muscle DNA synthesis in vitro and suppresses narrowing in arterial allografts and in balloon injured carotid arteries: Evidence that Rapamycin antagonizes growth factor action on immune and nonimmune cells. *Transplant Proc* 27:430–431, 1995
109. Gregory CR, Huang X, Pratt RE, *et al*: Treatment with Rapa-

mycin and MMF reduces arterial intimal thickening produced by mechanical injury and allows endothelial replacement. *Transplantation* 59:655–661, 1995
110. Geerling RA, De Bruin RWF, Scheringa M, *et al*: Suppression of acute rejection prevents graft arteriosclerosis after allogenic aorta transplantation in the rat. *Transplantation* 58:1258–1263, 1994
111. Pham SM, Shears LL, Kawaharada N, *et al*: High local production of nitric oxide as a possible mechanism by which Rapamycin prevents transplant arteriosclerosis. *Transplant Proc* 30:953–954, 1998
112. Shears LL, Kawaharada N, Tzeng E, *et al*: Inducible nitric oxide synthase suppresses the development of allograft arteriosclerosis. *J Clin Invest* 100:2035–2042, 1997
113. Tzeng E, Shears LL, Robbins PD, *et al*: Vascular gene transfer of human nitric oxide synthase: Characterization of activity and effects of myointimal hyperplasia. *Mol Med* 2:211–225, 1996
114. Morris RE, Huang X, Gregory CR, *et al*: Studies in experimental models of chronic rejection: Use of Rapamycin and Isosazole derivatives for the suppression of graft vascular disease and obliterative bronchiolitis. *Transplant Proc* 27:2086–2089, 1995
115. Meiser BM, Billingham ME, Morris RE: Effects of cyclosporin, FK506, and rapamycin on graft vessel disease. *Lancet* 338:1297–1298, 1991
116. Schmid C, Heemann U, Tilney NL: Factors contributing to the development of chronic rejection in heterotopic rat heart transplantation. *Transplantation* 64:222–228, 1997
117. Hasegawa S, Mitchell R, Nagano H, *et al*: Rapamycin reduces graft coronary arteriosclerosis and attenuates expression of IFNγ, MHC II and VCAM-1 in murine cardiac allografts. *J Heart Lung Transplant* 16:97, 1997
118. Wasowsksa B, Hancock WW, Onodera K, *et al*: Rapamycin and cyclosporin A treatment: A novel regimen to prevent chronic allograft rejection in sensitized hosts. *Transplant Proc* 29:333, 1997
119. Wasowska B, Wieder KJ, Hancock WW, *et al*: Cytokine and alloantibody networks in long term cardiac allografts in rat recipients treated with Rapamycin. *J Immunol* 156:395–404, 1996
120. Poston RS, Billingham M, Grant Hoyt E, *et al*: Rapamycin reverses chronic graft vascular disease in a novel cardiac allograft model. *Circulation* 100:67–74, 1999
121. Nicholson ML, Bailey E, Williams S, *et al*: Computerized histomorphometric analysis of protocol renal transplant biopsy specimens for surrogate markers of chronic rejection. *Transplantation* 68:236–241, 1999
122. Kliem V, Repp H, Frei U: Long term improvement of chronic renal graft dysfunction in Cyclosporin treated patients by addition of Azathioprine and reduction in Cyclosporin. *Transplant Proc* 26:2553–2555, 1994
123. Weir M, Anderson L, Fink JC, *et al*: A novel approach to the treatment of chronic allograft nephropathy. *Transplantation* 64: 1706–1710, 1997
124. Dimeny E, Fellstrom B, Larsson G, *et al*: Hyperlipoproteinaemia in renal transplant recipients: Is there a link with chronic vascular rejection. *Transplant Proc* 25:2065–2066, 1993
125. Fellstom B, Dimeny E, Larsson E, *et al*: Rapidly proliferative arteriopathy in cyclosporin induced permanently scarred rat cardiac allografts simulates chronic rejection. *Clin Exp Immunol* 80:288–292, 1990