PERSPECTIVES IN RENAL MEDICINE

What we CAN do about chronic allograft nephropathy: Role of immunosuppressive modulations

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What we CAN do about chronic allograft nephropathy: Role of immunosuppressive modulations. Given the potency of modern immunosuppressive agents, kidney transplantation across alloantingen barriers is a routine phenomenon with excellent 1-year graft survival in most centers. However, the improvement in 1-year graft survival has not been matched by improvements in long-term graft function and chronic allograft nephropathy (CAN) remains the second commonest cause of graft attrition over time. Calcineurin inhibitors, namely cyclosporine A (CyA) and tacrolimus, have been implicated as causal agents in the development of the fibrotic processes that are the hallmarks of CAN. Many studies have, therefore, concentrated on the improvement of long term graft function through the modulation of immunosuppressive therapy. It is the purpose of this review to describe and appraise the available evidence for the prevention and management of CAN through modulation of immunosuppressive agents.

Ever since the successful transplantation of human allografts between identical twins by Murray et al almost 40 years ago, solid-organ transplantation has been an impressive fusional achievement of scientific and surgical collaboration. Technical and pharmacologic advances, in particular the development and use of calcineurin inhibitors (CNIs), have made engraftment across alloantigen barriers routinely achievable with much reduced risk of acute rejection. Despite the recent introduction of new and expensive immunosuppressive agents, however, improvements in allograft lifespan have lagged behind those in 1-year survival (now routinely >90%) [1–4]. The two most common causes of long-term graft loss remain death with a functional graft, usually from a marked excess

of cardiovascular mortality in allograft recipients, and chronic allograft nephropathy (CAN), the term given to the development of fibrotic processes leading to progressive allograft dysfunction with variable proteinuria and hypertension [5, 6]. It has been proposed that these two processes may represent the systemic and local manifestations of developing (micro- and macro-) vascular disease that are accelerated in the presence of a functioning transplant and transplant immunosuppression. Indeed, recent studies have demonstrated that progressive injury to the renal microvasculature is among the first features of developing CAN [7] and that renal functional decline precedes the morphologic changes of CAN [8]. As such, amelioration of one may play an important role in improvement or prevention of the other, although randomized controlled intervention studies to test this hypothesis in renal transplantation have been scant [9].

The use of the term CAN, although convenient and much employed in the literature and in clinical practice, is a problem as it is a "catch-all" term. This observation is not just semantic pedantry. There are (at least) two processes, of different etiopathogenesis, that can occur to a variable extent, and which can be labeled "CAN" as the histopathologic findings overlap significantly. These are chronic cyclosporine A (CyA) nephrotoxicity, and chronic rejection. Interventions that may help the one may exacerbate the other. It is the latter which is classically identified (as in the Banff classification) by the histologic lesions of transplant glomerulopathy and arterial intimal thickening. Insufficient attention to date has been paid to understanding the relative contribution of each in an individual's case. Only recently have some important clues to differentiate these two processes emerged, and these need to be tested prospectively. These are peritubular/glomerular staining for C4d [10] and the production of collagen 1 [11]. In the former case this is associated with chronic transplant glomerulopathy (although this assertion is itself the subject of speculation [12]), and in the latter case chronic rejection. Further analysis of the difficulties in defining CAN fall outside the remits of this

Table 1. Risk factors for the development of chronic allograft nephropathy (CAN)

Alloantigen-dependent
  Acute rejection episodes
  Chronic subclinical rejection
Alloantigen-independent
  Age, gender, and race of donor
  Age, gender, and race of recipient
  Type of transplant (nonheart-beating > heart-beating cadaveric
    > live-related)
  Diabetes
  Hypertension
  Hyperlipidemia
  Length of time on dialysis
  Ischemia/reperfusion injury
  Cytomegalovirus infection
  Drugs, in particular calcineurin inhibitors

Both alloantigen-dependent and alloantigen-independent mechanisms are thought to play a part in the pathogenesis of the condition (synthesized from [12 to 19]).

article but it is important to note that the definitions for CAN are not universal and that studies are therefore difficult to compare.

Although various risk factors for the development of CAN have been identified, its pathogenesis is incompletely defined (for an excellent article on this subject, please refer to [13]). Both alloantigen-dependent and alloantigen-independent mechanisms are thought to play a part in the pathogenesis of the condition [12–19] (Table 1). Pilmore and Dittmer's study, reported in 2002, showed that chronic CyA toxicity (defined as extensive arteriolar hyalinosis and obliteration), though frequently colocalizing with glomerular and interstitial fibrotic changes, was a separate entity, and although renal function deteriorated progressively in patients with or without chronic vascular changes, those patients with chronic vasculopathy due to CNIs responded almost uniformly favorably to reduction in calcineurin inhibitor exposure [22].

A recent paper from Jeremy Chapman's group in Australia, based on information from sequential protocol biopsies, showed that there were two distinctive phases of allograft injury. The first of these is a composite of ischemic injury, prior acute rejection, and subclinical rejection. The second, usually more than 12 months postengraftment, is allograft injury characterized by high-grade arteriolar hyalinosis, glomerulosclerosis, and tubulointerstitial scarring. In this study, additional tubulointerstitial damage was found to accompany the use of CNIs [5], which corroborated a recent analysis of 40,963 first kidney transplant recipients between 1987 and 1996 from the United States Renal Data System (USRDS). This latter paper identified a more rapid rate of decline in renal function in patients on chronic CNI therapy [23], presumably through mechanisms that either cause or exacerbate CAN.

Because of the concerns that prolonged patient exposure to calcineurin inhibitors was exacerbating or promoting CAN [23, 24] there have been many trials in which the aim has been to reduce CNI exposure. These have included de novo avoidance of calcineurin inhibitor use, phased calcineurin inhibitor withdrawal without drug additions, substitution of one calcineurin inhibitor for another, or the use of azathioprine, mycophenolate mofetil (MMF), or sirolimus as calcineurin inhibitor–sparing agents.

It is the purpose of this review succinctly and critically to describe and appraise the available evidence for the prevention and management of CAN through modulation of immunosuppressive agents. As such, we hope to guide the practicing transplant physician or surgeon faced with the difficult clinical decisions necessary to help patients with CAN. Central to our analysis of the available options will be three criteria: (*1*) the success of the intervention at arresting renal function decline/rate of allograft attrition, (*2*) the side-effect profile of any new immunosuppressive agents added, and (*3*) the rate of acute or chronic rejection (and the graft loss therefrom), or acute infectious complications, as a result of changes in the overall potency of immunosuppression.

CALCINEURIN INHIBITORS

From their inception, CNIs have brought about a remarkable reduction in transplant rejection and excellent one-year graft survival. However, this has come at the price of long-term graft dysfunction as the processes underlying CAN seem intimately related to their use [23–25]. The most common approach to prevent development of CAN or to ameliorate it once developed is to reduce exposure to CNIs by one of two methods, either to reduce the delivered dose, which carries a risk of precipitating acute rejection, or to add in a different immunosuppressant while reducing or eliminating the calcineurin inhibitor (so as to minimize the risk of acute rejection). The study of Pascual et al [26] was instructive in demonstrating that a 50% reduction in delivered dose of CNIs in patients with stable renal function did not result in a higher rate of acute rejection episodes compared to an equal number of similar patients ($N = 32$) randomized to continue same-dose CNI after 6 months of followup. However, those randomized to dose reduction did show improved blood pressure and lipid profiles. Similarly, the randomized controlled trial of Abramowicz et al [27] indicated that withdrawal of CyA from a triple immunosuppression protocol (MMF, steroid, and CyA) can result in improved renal function and cardiovascular profile at a cost of a modest increase in reversible acute rejection episodes (nine versus two subjects in withdrawal and control arms, respectively) by the end of 6 months of follow-up. However, in the setting of preventing CAN

or of treating it once developed there is actually very little evidence to support simple dose reduction in CNIs without the addition of another agent. Perhaps the closest is the study by Pilmore and Dittmer [22] from New Zealand, which looked at 46 biopsies from patients who had been transplanted for more than 6 months. Patients were categorized into those with CAN alone ($N = 16$), those with a combination of CAN and calcineurin inhibitor nephrotoxicity (CNIN; $N = 21$), and those with neither nephrotoxicity nor CAN ($N = 9$). Patients with evidence of nephrotoxicity had a significant dose reduction in CNI while those with CAN alone had no change in therapy. After 17 months of follow-up, it was found that patients with CNIN had a rapid improvement in renal function within 1 month after dose reduction which was sustained for the duration of follow-up, while those with CAN alone had a gradual decline in renal function. Acute rejection was not precipitated by the CNI withdrawal. Unfortunately, this study did not incorporate dose reduction of CNI in CAN without active nephrotoxicity; therefore, it cannot differentiate whether the improvement in graft dysfunction was the result of improvement in the nephrotoxicity or of the CAN component (sometimes a genuinely difficult clinical distinction to make).

CYCLOSPORINE AND TACROLIMUS: WHICH CNI IS BETTER FOR CAN?

With regards to type of calcineurin inhibitor, there are theoretical advantages of tacrolimus over CyA. Among these, primary immunosuppression with a tacrolimus-based regimen is associated with better cardiovascular risk profile than a CyA-based regimen in nondiabetic patients [28] (although there is no evidence that this improvement translates to reduced cardiovascular events) and results in fewer and less severe acute rejection episodes [29]. These advantages are reviewed in detail elsewhere [30] but have prompted investigation into substitution of tacrolimus for CyA. Studies can essentially be divided into those comparing the effects of tacrolimus versus CyA as primary immunosuppression on the propensity to develop CAN and those comparing a secondary switch from CyA to tacrolimus once CAN has developed. Murphy et al [31] carried out a prospective randomized trial using either tacrolimus or microemulsion CyA (Neoral) together with azathioprine in 102 nonheart-beating renal allografts. At 1 year, transplant interstitial fibrosis was quantified using computerized histomorphometric measurement of picro sirius red-staining on protocol biopsies. There was a significant increase in allograft interstitial fibrosis in the patients treated with Neoral compared with those given tacrolimus in the absence of differences in acute rejection episodes, steroid-resistant rejection, or pretransplantation risks for CAN. This study was corroborated by

that of Jurewicz [32], who analyzed 6-year follow-up data from 232 renal transplant recipients randomized to treatment with tacrolimus or CyA. Renal function, as determined by the glomerular filtration rate (GFR), was significantly better in tacrolimus-treated patients from month 3 posttransplant and normal renal function was maintained throughout a 5-year follow-up in a significantly higher proportion of nonrejecting patients treated with tacrolimus than with CyA (58% versus 10%, respectively, at 5 years; $P = 0.002$). As with the previous study, analysis of protocol biopsies revealed that the degree of interstitial fibrosis, similar in both treatment groups at baseline, was significantly greater in the CyA group after 12 months. Furthermore, they found that patients receiving tacrolimus had significantly greater 6-year graft survival (81% versus 60%; $P = 0.0496$) and a higher projected graft half-life (15 versus 10 years) than those receiving CyA. Moreover, both studies identified better lipid profiles and cardiovascular risk in the tacrolimus cohort patients than their CyA-treated counterparts.

A recent report from Turkey blindly scutinizing protocol biopsies carried out in the first 6 months posttransplant in 35 patients randomized to either tacrolimus or CyA concluded that, although the incidence of acute rejection episodes may not be significantly different between the two groups, subclinical acute rejection and subclinical CAN was more common with CyA than tacrolimus [33] (although their findings did not reach statistical significance, possibly in view of the small sample size). These differences in histology may well be explained by the differential expression of genes encoding extracellular matrix material in transplant glomeruli between patients given tacrolimus versus those given CyA as primary immunosuppression. Indeed, Bicknell et al [34] have shown a persistent increase in mRNA encoding type II collagen and tissue inhibitor of matrix metalloproteinase 1 and 2 (TIMP-1 and TIMP-2) [but not transforming growth factor-β1 (TGF-β1)] in 51 transplant biopsies of patients on CyA compared to those on tacrolimus from as early as the first week posttransplant. Some of their early findings could undoubtedly be accounted for by differences in biopsy material and the source of the transplant (living or cadaveric donor). However, the same observations were made on the 6-month protocol biopsies, making it likely that the expression of matrix components is more favored by the presence of CyA than tacrolimus.

Unfortunately, all these studies have been carried out over a relatively short period of time and with small patient numbers. Therefore, the likelihood of deriving a beneficial result by chance alone is relatively high. Furthermore, there is no guarantee that histologic studies carried out at 6 months or a year will correlate with the development of CAN over the next few years. These problems are highlighted by a detailed multivariate analysis of

the USRDS, which demonstrated comparable 3-year graft survival for both cadaveric and living donor renal transplant patients receiving either CyA-Neoral or tacrolimus with MMF and steroids, with no significant differences between treatment groups. They included 9449 patients in the multivariate analysis (2130 on tacrolimus and 7319 on CyA) pooling data from 1995 to 1998 [35]. Similarly, the FK506 Kidney Transplant study Group failed to demonstrate a difference between CyA and tacrolimus in the likelihood of developing CAN on protocol biopsies carried out at 2 years postengraftment in 144 subjects [36].

Substituting tacrolimus for CyA once CAN has developed is another therapeutic option. Results for this approach have been mixed and there was, in general, a paucity of data in the literature until very recently. An early, cross-sectional pilot study by Jurewicz [37] followed 14 patients with biopsy-proven CAN for 15 months after a switch from CyA to tacrolimus. They reported two distinct responses. One group, comprising nine patients, demonstrated continued deterioration in the estimated GFR, while the second group, of five patients, showed an improvement (who extended their return to dialysis by a median of 41 months). In contrast, the trial conducted by Stoves at al [38] from the United Kingdom showed that substitution of tacrolimus for CyA in patients with biopsy proven CAN and declining renal function had no advantage over continued CyA over a 6-month follow-up period [36], although it could be argued that the length of follow-up was insufficient and that the study, comprising 14 patients in each group, was underpowered to detect small differences between them.

More recently, the group of Lee et al [39] retrospectively analyzed a heterogeneous cohort of 34 renal transplant recipients who had had biopsy proven acute cellular rejection or CAN and who had been switched from CyA to tacrolimus. Up to 72 months of follow-up later, a consistent pattern of improved renal function and slowing of renal functional decline was observed in comparison to baseline. Blood pressure control was also improved. Also very recently, the publication of Waid et al reported on a group of 186 subjects fulfilling clinical criteria for CAN (90% with baseline biopsy demonstrating CAN) who had had a switch from CyA to tacrolimus in a 2:1 ratio. After 2 years of follow-up, serum creatinine was significantly better in the tacrolimus treated cohort (which was better than at baseline) while acute rejection episodes and allograft survival was similar. A further 3 years of follow-up is planned for these patients [40]. A similar but smaller study, unsupported by renal biopsy, comprising 30 subjects, demonstrated comparable results up to three years of follow-up [41].

Given the small patient numbers in the trials highlighted above and the clinical heterogeneity of patients in some of them, it is difficult to be certain whether substitution of tacrolimus for CyA will reduce the risk of CAN in the long term and whether this switch may be of benefit for secondary prevention. The body of evidence for the latter approach has been increasing in the recent past but the majority of studies have, quite rightly, concentrated on substituting a noncalcineurin inhibitor for CyA.

AZATHIOPRINE

Azathioprine has long been used as a steroid sparing agent in a variety of clinical scenarios. Switching from CyA to azathioprine in stable renal allografts has previously been demonstrated to improve cardiovascular risk profile and incidence of gout [42, 43]. The natural postulate is whether it can be used as a calcineurin inhibitor–sparing agent in order to reduce the incidence of CAN. One animal study using Fisher kidneys transplanted into bilaterally nephrectomized Lewis rats (i.e., high immunogenic risk) [44] employed a CyA-based induction protocol followed by switch at day 11 to either CyA and prednisolone, azathioprine and prednisolone, or vehicle and prednisolone. Organs harvested at 24 weeks for morphology and immunohistochemistry, however, demonstrated no difference between the CyA or azathioprine-based immnosuppression protocols in the development of changes of CAN. It is, however, difficult to be certain as to what extent this model reflects the human disease and difficult to know whether the length of exposure to the various agents was sufficient to demonstrate a difference between them. Furthermore, groups consisted of only eight rats each, such that heterogeneity in immune responsiveness between individuals could well have skewed the results.

Azathioprine is a less potent immunosuppressive agent than CyA; there was therefore a higher incidence of acute rejection in the pre-CyA era using azathioprine-based protocols. The trial of MacPhee et al [45] demonstrated that azathioprine can be used in patients with stable allograft function as early as the first year posttransplantation to permit CNI withdrawal with the benefit of improved graft function but at a cost of increased acute rejection episodes in the first few months postswitch. As such, human data comparing the risks of developing CAN using azathioprine as primary agent versus CyA are scant. However, there are several historic studies using azathioprine to modify the risk of developing "CyA toxicity" and "chronic rejection" in existing CNI–based regimens. Unfortunately, many of these studies were carried out in the pre-Banff era, so it is difficult to be certain as to the lesional correlation between "chronic rejection" and what was later defined as CAN. Furthermore, without classification of severity, it is possible that their populations of "chronic rejection" could have been of low severity and therefore more likely to respond to treatment. Sweny et al [46] randomized a cohort of 77 nondiabetic stable

cadaveric renal transplants at 1 year to either convert from CyA to azathioprine or to continue CyA. Patients were subsequently followed up for 12 months. An improvement in reciprocalized serum creatinine and an improvement in blood pressure control were noted in the 33 patients randomized to conversion. However, nine of these individuals experienced acute rejection, of whom six returned to CyA. On the other hand, six patients randomized to continue CyA had to switch to azathioprine as a result of CyA toxicity. CyA levels remained unchanged in those continuing CyA but serum creatinine levels demonstrated a progressive decline with time. Pascual et al [47] added azathioprine to an ongoing CyA-prednisone protocol in 31 patients without reducing dose of CyA. Subjects were a mean of 11.3 months after renal transplantation and were subsequently followed up for a mean of 23 months. Patients were split into three groups: those with "chronic rejection," those with repeated episodes of acute rejection, and those with CyA toxicity despite dose reduction. In the first group, serum creatinine had risen over the 6 months prior to azathioprine (renal function declining at a rate of -0.13 ± 0.12 creatinine^{-1}/month) but improved at a rate of 0.05 ± 0.07 creatinine^{-1}/month in the 6 months postazathioprine and at a rate of 0.05 ± 0.12 creatinine^{-1}/month during the entire follow-up period ($P < 0.01$); CyA levels remained stable. The second group had a greater decline in renal function but this was arrested after addition of azathioprine. In group three, renal function improved in eight patients. At the end of the study, 15 patients had improved graft function, two were stable, 12 had worsened (nine on dialysis), and two had died. It is interesting to note the improvement of allograft dysfunction in all three groups simply by the addition of further immunosuppression and rather surprising in the last group with CyA toxicity. Unfortunately, the results of this study have never been repeated.

More recently, Bakker et al [48] carried out an open-labeled randomized trial of conversion from CyA to azathioprine at 3 months posttransplant with 128 patients allocated to either continued CyA or switch to azathioprine. At 2 years posttransplant, graft survival and GFR were already significantly better in those on azathioprine and the risk of developing CAN was lower [relative risk for CyA 4.3 (95% CI 1.4 to 12.9) ($P = 0.009$)]. Furthermore, more biopsies from those on CyA showed features of nephrotoxicity which prompted a late switch. At 15 years of follow-up, graft survival was higher in the azathioprine arm (76.5% versus 64.7%), although this did not reach statistical significance until the data were analyzed according to patients staying on assigned treatment (i.e., not by intention-to-treat). Acute rejection rates were scarce in this context but many fewer patients were on blood pressure and lipid-lowering medications at the end of the study. Unfortunately, the small patient numbers

and failure to demonstrate a significant difference in graft survival by intention-to-treat analysis argue that the difference between CyA and azathioprine on the basis of this trial was actually quite small. Indeed, conversion to azathioprine carries an increased risk of early rejection [49], which is why patients enrolled into this study also had a temporary increase in steroid dose to cover the transitional period.

With regard to using azathioprine to treat "chronic CyA nephropathy," the study of Mourad et al [50] followed up a cohort of 23 patients with biopsy-proven chronic CyA nephropathy who were given azathioprine and had either dose reduction (18 of 23) or cessation (5 of 23) of CyA. At the end of 2 years follow-up, they observed a significant improvement in GFR (mean 40 to 47 mL/min) with concomitant improvement in serum creatinine. Blood pressure, likewise, improved. One episode of reversible acute rejection was documented.

Dosing of azathioprine is generally done by subject weight (and not by drug levels) and its effects are governed by metabolism to its active metabolite 6-mercaptopurine (6-MP) via the thiopurine methyltransferase (TPMP) enzyme system. Its clinical efficacy and adverse effects profile correlate very well with tissue levels of 6-MP, although some idiosyncratic reactions have also been known to occur. There is more than 40 years of experience with this drug, and generally it is very well tolerated.

On the basis of a propensity to precipitate acute rejection and the weaknesses of trials highlighted above, it would appear then that azathioprine is less than ideal as a CNI–sparing agent. In addition, the lack of published negative data (which is suggestive of the process of publication bias) and the paucity of further positive trials may indicate that the effects of azathioprine on developing CAN could be much worse than the available literature is currently suggesting. However, more potent immunosuppressants have since emerged and have prompted similar investigations.

MMF

MMF is a more potent immunosuppressive agent than azathioprine [51] and has supereded azathioprine in many centers for primary immunosuppression in combination with a CNI and steroid [52]. It acts as a prodrug whose active metabolite inhibits the activity of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the generation of purines in the S phase of the cell cycle. As such, it has an antiproliferative effect (e.g., on smooth muscle cells) that may be useful in preventing or treating the scarring processes underlying CAN. Additionally, MMF inhibits the expression of adhesion molecules on endothelial cells, thereby retarding infiltration by inflammatory cells [53], increases apoptosis of T lymphocytes

[54], and reduces antibody production from B cells [55]. Indeed, a prospective randomized trial by Merville et al [56] evaluating the likelihood of developing biopsy-proven CAN at 1 year in patients on a CyA-based protocol randomized to include either azathioprine or MMF demonstrated that the incidence of CAN was significantly lower in patients on MMF (31% versus 63%). CyA levels were the same between the two groups. Therefore, on the assumption that calcineurin-free protocols would reduce the incidence of CAN through primary prevention and in vitro experiments showing that MMF inhibits activation of human mesangial cells [57], several studies have looked at the use of MMF as a calcineurin-sparing agent for primary prevention of CAN. Initially, Vincenti et al [58] carried out a multicenter, open-label, cohort study using a protocol of daclizumab, MMF, and corticosteroids. A total of 98 primary kidney transplant recipients of low immunologic risk were given 2 mg/kg daclizumab before transplantation and 1 mg/kg for a fortnight (for a total of five doses), MMF 3 g/day for 6 months followed by 2 g/day thereafter and conventional corticosteroid. Almost 50% of patients experienced an episode of biopsy-proven acute rejection by 6 months. Nevertheless, 1-year graft and patient survival were still 97% and 96%, respectively, at a cost of initiating calcineurin inhibitors in 62% of the patients as a result of acute rejection. Mean serum creatinine at 1 year was 113 μmol/L (1.57 mg/dL) in nonrejectors and 154 in patients who experienced rejection and were then started on a calcineurin inhibitor. At 1 year, 16% of patients who had protocol biopsies showed histologic evidence of CAN. These patients showed significantly lower expression of TGF-β, fibronectin, and collagen than a cohort of CNI–treated historic controls. Tran et al [59] conducted a very similar prospective, nonrandomized, open-label trial using the same agents in favor of CNI in 45 kidney transplant recipients. The immunsuppression protocol was identical to the previous study except for tapering prednisolone to 15 mg/day instead of 10 mg/day at 6 months. Again, there was a high incidence of biopsy-proven acute rejection (approximately one third of patients) although graft and patient survival remained excellent (95% and 100%, respectively). CyA was eventually started on 51% of patients as a result of acute rejection or intolerance of MMF or steroids. These individuals had a higher serum creatinine [mean 168 versus 106 μmol/L (1.9 versus 1.2 mg/dL)] and required more antihypertensive medications.

Early conversion at 3 months from a CyA-based regimen to a CyA-free regimen using MMF and steroid as maintenance treatment was subsequently assessed by an open randomized trial by Schnuelle et al [60]. Their cohort of 84 renal transplants converted at 3 months posttransplantation showed better creatinine clearance (71.7 versus 60.9 mL/min) and calculated GFR (73.2 versus 61.9 mL/min) at the end of 1 year in comparison to those

subjects randomized to continue CyA. However, acute rejection episodes were again found to occur with greater frequency after withdrawal of CyA (11.3% versus 5.0%).

Although these papers are encouraging in that they provide histologic evidence of reduced expression of fibrogenic genes (albeit in very small numbers of patients) and demonstrate that a proportion of patients can be maintained CNI–free, they lack control data and the element of randomization. Furthermore, the high incidence of acute rejection is unsatisfactory and could actually increase the risk of developing CAN in the longer term, particularly since most of the subjects recruited into these trials were of low immunogenic risk.

The risk of acute rejection, however, reduces with time, permitting dose reduction or even cessation of certain immunosuppressants. The question, then, is whether MMF can be added as an adjunct into a CNI–based regimen to allow dose reduction or cessation of the CNI without precipitating acute rejection and whether this maneuver results in improved graft survival. Several studies have now demonstrated that the introduction of MMF and the reduction or withdrawal of CyA have a favorable outcome in the setting of CAN (see Table 2). Although there are animal experiments in abundance [61], the first significant clinical description in the literature of altering the immunosuppressive regimen involving MMF to treat CAN was by Weir et al in 2001 [62]. They studied 118 patients with declining renal function and biopsy-proven CAN in whom CNIs were reduced in 100 and discontinued completely in 18, and in whom MMF was initiated at a dose of 2 g/day [although, in the event, the eventual administered doses were mostly in the range 1.2 to 1.5 g daily (for largely unspecified reasons)]. At a mean follow-up of 651 days after the intervention, improvement in renal function was evident in the majority of patients as judged by amelioration of slopes of reciprocalized serum creatinine or lack of deterioration in the slopes. The intervention was well tolerated and episodes of acute rejection were scant.

Francois et al [63] recently reported a controlled study of the use of 2 g daily MMF in 39 patients with CAN using conventionally treated patients as controls. Although there was an improvement in serum creatinine in the switch group at 1 year and 3 years in comparison to baseline (the control group showed no change in creatinine over time), graft survival was comparable between the two groups. Furthermore, they noted a high incidence of abdominal symptoms, systemic infections, and anemia, all signs of excessive immunosuppression with MMF, which led to discontinuation in two case and dose reduction in 18. This trial was mirrored by that of Ducloux et al [64] whose regimen of substituting MMF for Aza and withdrawing CyA in 31 patients with CAN demonstrated improved serum creatinine after conversion, which remained stable after a mean follow-up of 27 months

Table 2. Trials using mycophenolate mofetil (MMF) in chronic renal allograft nephropathy (CAN) with reduction/complete withdrawal of calcineurin inhibitors (CNI)

Author	Publication	Patient number	MMF dose g/day	Result	Commentary
Weir et al	*Kidney Int* 59:1567–1673, 2001	118	Intention was 2.0; in practice was 1.2 to 1.4	92% of no CNI group and ~55% of reduced CNI group improved by 1/creatinine	Benchmark study, 100 CNI reduced, 17 ceased 651 days follow-up, MMF "well tolerated"
Afzali et al	*Transplantation* 79:304–309, 2005	89	1.0	Stepwise improvement in mean 1/creatinine after 12 months, sustained over the next 2 years of follow-up	Very well-tolerated with only 7/89 subjects discontinuing MMF due to side effects, only one acute rejection episode over first 12 months
Dudley et al	*Transplantation* 79:466–475, 2005	144	2.0	Stepwise improvement in mean 1/creat over 12 months with deterioration in the CyA group	85% incidence of adverse effects with 2 g of MMF, no acute rejection episodes
Kerecuk L et al	*Pediatr Nephrol* 2005 (Epub August 16)	19	Mean 1.0 g/m^2	Improvement in renal function, graft rejection rate, and BP. Well tolerated.	Pediatric study. Relatively short follow-up. Not in print yet.
Suwelack et al	*Am J Transplant* 4:655–662, 2004	39	Intention was 2.0; in practice was 1.3 to 1.6	Withdrawal of CNI resulted in improvement in slope of 1/creatinine at 32 weeks follow-up; blood pressure better off CNI at 35 weeks follow-up	No acute rejection episodes, generally well tolerated but high incidence of anemia
Francois et al	*Nephrol Dial Transplant* 18:1909–1916, 2003	39	2.0 (maintenance dose of 1.1)	↓ in mean creatinine from 192 to 172 μmol/L (2.16 to 1.94 mg/dL) at 1 year ($P = 0.004$) and 159/μmol/L (1.79 mg/dL) at 3 years ($P < 0.003$)	Control group high incidence of side effects (e.g., infections and anemia)
Yeung at al	*Transplant Proc* 35:176–178, 2003	9	1.0 to 2.0 (mean 1.4 ± 0.2)	Comparison of postconversion 1/creatinine to preconversion 1/creatinine slopes showed significant improvement in rate of decline in renal function ($0.74 \pm 3.16 \times 10^{-3}$ vs. $-2.71 \pm 3.68 \times 10^{-3}$)	No side-effects reported, small numbers
Ducloux et al	*Transplant Int* 15:387–392, 2002	31	2.0	↓ in creatinine from 227 ± 31 to 185 ± 50 μmol/L (2.56 ± 0.35 to 2.08 ± 0.56 mg/dL) ($P < 0.0005$)	29% acquired systemic infections requiring hospitalization
Khachatryan et al	*Transplant Proc* 34:807–808, 2002	38	10 patients, 1.0; 17 patients, 1.5; 11 patients, 2.0	Creatinine slope for month −6 to 5, 3.085; creatinine slope for month 5 to 12, −0.879	Renal function improved after 5 months of starting MMF, patient only tolerated dose of 1.5 g/day
Falkiewicz et al	*Transplant Proc* 34:567–568, 2002	38	2.0	No significant change in creatinine after 6 months post-MMF compared with increased creatinine by 42% in group without MMF introduction	Conversion to MMF inhibited decline in renal function
Noronha et al	*Transplant Proc* 34:491–493, 2002	20	2.0	Mean 1/creatinine slope in control group similar to MMF group	Difference between MMF and control survival curves statistically significant
Gonzalez Molina et al	*Transplant Proc* 34:335–337, 2002	122	2.0	Slope of 1/creatinine before and after MMF introduction, −0.0002 and −0.000007 ($P < 0.001$)	Shows reduction in kidney function impairment with MMF and even some improvement
McGrath et al	*Transplant Proc* 33:2193–2195, 2001	15	MMF started at 1.0, increased to 2.0	Average creatinine reduction from 246 to 188 μmol/L (2.77 to 2.12 mg/dL) at 6 months in MMF group ($P < 0.001$)	
Henne et al	*Transplantation* 76:1326–1330, 2003	36	1.2 mg/m^2	61% showed a rise of 7.5 mL/min in glomerular filtration rate at 1 year	Children, 36% abdominal pain, 6% anemia

CyA is cyclosporine A; BP, blood pressure.
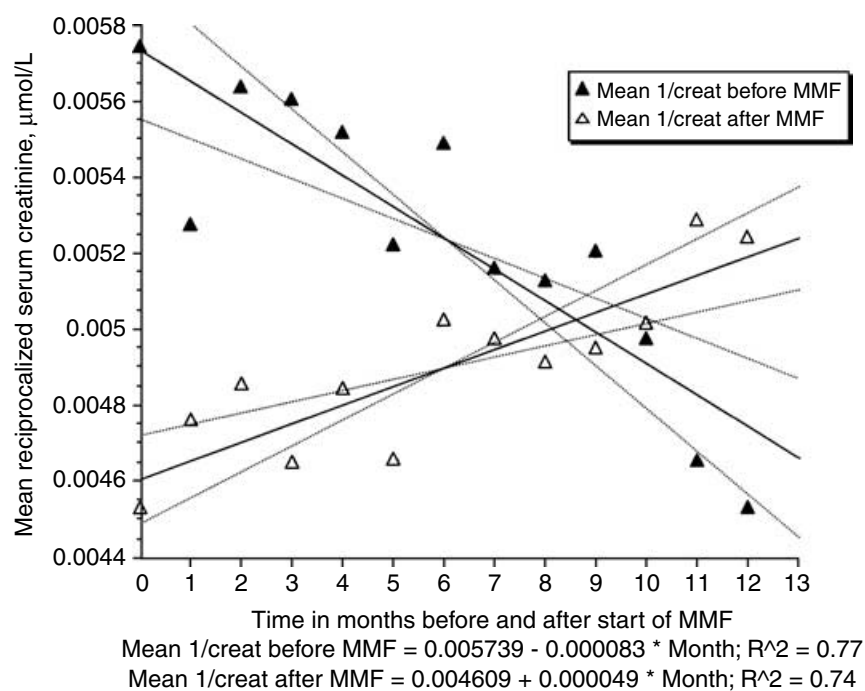
Fig. 1. **Mean reciprocalized serum creatinine (1/creatinine) before and after introduction of mycophenolate mofetil (MMF) over a 12-month run-in and follow-up period.** A total of 89 patients with established chronic renal allograft nephropathy (CAN) were started on a regimen of MMF at a median dose of 1 g/day with phased reduction in delivered dose of CNIs over a 12-month period. The 95% confidence bands for the regression lines are depicted. This dose was very well tolerated and only 7 out of 89 patients stopped MMF as a result of adverse effects. Only one episode of acute rejection was observed.

[227 ± 31 μmol/L versus 185 ± 50 μmol/L (2.55 ± 0.35 versus 2.01 ± 0.56 mg/dL; $P < 0.0005$)]. However, they also reported a high incidence of infection (28% of patients) with the 2 g daily dosing regimen, an observation that was repeated by Khatchatryan et al [65], who found that it was difficult to exceed 1.5 g/day of MMF without troublesome side effects. This difficulty was also evident in the recently reported and elegantly designed "creeping creatinine study," where 144 subjects with CAN were randomized to two groups. The first group was initiated on a combination of steroid and MMF titrated up to 2 g with elimination of CyA, while the second group continued CyA as previously. Patients in the first group showed a significant stepwise improvement in 1/creatinine at 6 months and maintained this improvement to the end of the study at 12 months, while subjects in the second group continued to show deteriorating graft function. Two grafts in the first group and four in the second were lost during the study period but there were no acute rejection episodes. Unfortunately, there was an 85% incidence of adverse events with a daily dose of 2 g of MMF, particularly gastrointestinal, infective, and hematologic events [66]. Our own observations aimed at elucidating the effectiveness and tolerability of lower doses of MMF (mean of 1 g/day) over the long term have shown that lower doses of MMF are very well tolerated without precipitating episodes of acute rejection and lead to improved graft function that are maintained in the long term [67] (Fig. 1).

Although the above studies nearly all demonstrate improved graft function and/or graft survival, a number of caveats exist. First, most studies have been carried out in small numbers of patients, without the element of randomization and over short periods of time. The study of Weir et al, who demonstrated good tolerance of MMF consisted of a majority of African Americans, whose immunogenetics are known to be different to other populations, so the recorded responses may not necessarily be comparable to those of other cohorts. The lack of good meta-analyses is a demonstration of the heterogeneity of study protocols and the difficulty in translating data from these small patient populations to larger, different ones. Furthermore, it is unclear whether it is the addition of MMF per se which results in improved outcome or the reduction/withdrawal of CNI. This latter question has in part been addressed by two studies, carried out by Henne et al [68] and the Spanish Cooperative Study Group of Chronic Allograft Nephropathy [69]. The report by Henne et al in children with CAN exposed to 1.2 mg/m^2 of MMF without a change in CNI level showed a favorable allograft functional outcome at the price of significant (mainly gastrointestinal) side effects. Their cohort of 36 children showed no significant change in trough CyA levels at 1 year (114 ng/mL before and 98 ng/mL at 1 year after) [68]. The Spanish study recruited 121 patients with biopsy-proven CAN, 59 of whom were on treatment with CyA and prednisolone and 62 of whom were receiving CyA, prednisolone, and azathioprine. Each group was given 2 g per day of MMF and azathioprine was stopped. Renal function as judged by Cockcroft-Gault GFR remained stable during the (median) 36 months of follow-up while the slope of the GFR improved. In 65 patients whose CyA levels had remained unchanged

during follow-up, there was a reduction in the rate of loss of GFR [69]. Both of these studies are suggestive that the addition of MMF was responsible for ameliorating transplant function since the CyA levels and exposure remained unchanged. However, both studies consisted of small numbers of subjects and the latter's findings were the result of a subanalysis, which would increase the likelihood of deriving a statistically significant result by chance alone. One additional complexity of altering CNI dosage in the context of MMF is the complex pharmacokinetic interactions between these classes of immunosuppressive drugs. The efficacy and side-effect profile of MMF is related to trough mycophenolic acid (MPA) levels [70]. There are significantly lower levels of plasma MPA for the same MMF dosage in patients taking CyA compared to patients on tacrolimus [69]. Given these interactions it would seem advantageous for MMF doses to be determined by MPA blood levels in future trials. Additionally, the findings of these two studies is refuted by those of Suwelack et al [71], who carried out the only trial to date of adding in MMF to a CNI–based regimen in the context of established CAN and then randomizing patients to either withdrawing or continuing with the CNI. The trial was stopped prematurely as a significant difference in renal function was found in an interim analysis which strongly favored the withdrawal of CNI.

A reasonable number of small studies have so far provided data for the use of MMF to allow dose reduction or cessation of CNIs in the setting of CAN and the data are on the whole quite encouraging. Nevertheless, one must bear in mind the possibility of publication bias, particularly since nearly all available investigators have reported positive findings. Furthermore, these findings have not yet been subjected to a large multicenter randomized, double-blinded trial due to the unethical nature of carrying out a placebo-controlled study in this setting and the difficulty in achieving double blinding (given that drug levels will need to be monitored).

SIROLIMUS

Sirolimus acts by binding to FK-binding protein (FKBP) and the SRL-FKBP complex then inhibits mTOR (mammalian target of rapamycin) and by so doing blocks the downstream signal transduction pathways required for progression of a cell from G_1 to the S phase of the cell cycle. It thus acts as a potent immunosuppressive and antiproliferative agent with the properties of inhibiting fibrogenesis, a function that maybe useful in preventing the scarring processes observed in CAN. The advent of sirolimus has, therefore, prompted renewed enthusiasm in primary prevention of CAN by using sirolimus-based regimens to avoid calcineurin inhibitors (Table 3). Animal experiments have highlighted its ability, in com-

bination with MMF, to prevent the features of CAN, namely fibrous intimal thickening, allograft glomerulopathy, and interstitial fibrosis [72, 73]. As a result, a randomized open-label, multicenter trial recruited 78 cadaveric renal allograft recipients of low immunologic risk and randomized them to treatment with sirolimus or CyA, in combination with MMF and steroids [74]. Sirolimus was administered to achieve a level of 30 ng/mL for the first 2 months and 15 ng/mL thereafter, while CyA was dosed to achieve trough levels of 200 to 400 ng/mL for 2 months and 100 to 200 ng/mL after that. The dose of steroids and MMF was the same in both groups but MMF was discontinued at 6 months for both groups with an option to convert to azathioprine if needed. They identified no significant difference in patient and graft survival or in biopsy-proven acute rejection after 12 months of follow-up. However, from 2 months onwards, calculated GFR was consistently higher in the sirolimus arm. A higher incidence of hypercholesterolemia, hypertriglyceridemia, thrombocytopenia, and diarrhea was found in the sirolimus-treated patients, the latter two being adverse effects of MMF (sirolimus increases levels of MPA). Very similar results have previously been published by the Sirolimus European Renal Transplant Study Group, which compared sirolimus to CyA for primary immunosuppression, in combination with azathioprine and steroid [75] using virtually identical protocols. Indeed, the 2-year results of these two trials were later analyzed together and demonstrated that serum creatinine was consistently lower in sirolimus-treated groups who had a calculated GFR that was on average 10 mL/min better [76]. Even aiming for lower levels of sirolimus and CyA as in the study of Flechner et al [77] (target level of 10 to 12 ng/mL for 6 months and 5 to 10 ng/mL thereafter for sirolimus and 200 to 250 ng/mL for CyA) but using induction therapy with basiliximab, no difference in acute rejection episodes or graft survival was found between sirolimus and CyA groups but sirolimus-treated patients had better calculated GFR (81.1 ± 23.9 mL/min versus 61.1 ± 14.6 mL/min; $P < 0.01$). Adverse effect profiles in this study were not different. The observed beneficial effects of this therapy were consistent in these patients who were then reanalyzed at 2 years postengraftment. GFR remained significantly better in the sirolimus arm and, of the 48 patients who were rebiopsied, 67% versus 21% had normal (Banff 0) biopsies when compared to the CyA arm [78].

One other option is to transplant as per current protocols using a CNI and, once the early rejection-prone period is over, to convert from CNI to sirolimus in order to reduce the long-term probability of developing CAN. The multicenter trial of Johnson et al [79] used CyA in combination with low dose sirolimus as primary immunosuppression and then randomized subjects to either

Table 3. Randomized controlled trials comparing sirolimus-based and calcineurin-based immunosuppression after renal transplantation, including studies of calcineurin inhibitor (CNI) reduction/withdrawl

Author	Publication	Study design	Outcomes	Commentary
Groth et al	*Transplantation* 67:1036–1042, 1999	82 randomized to sirolimus/steroids/azathioprine or CyA/steroids/azathioprine; target sirolimus level 30 ng/mL in first 2 months posttransplant, then 15 ng/mL thereafter	No significant difference in patient survival, graft survival, or biopsy confirmed acute rejection at 1 year; GFR significantly better ($P < 0.05$) in sirolimus group at 12 and 16 weeks posttransplant, but no significant difference at 1 year	At 1 year, 24 of the 41 patients in the sirolimus group had sirolimus discontinued, including 12 patients for acute/borderline rejection. Only 6 of the 42 patients in the CyA group had CyA discontinued for acute rejection
Kreis et al	*Transplantation* 69:1252–1260, 2000	78 randomized to sirolimus/MMF/steroids or CyA/MMF/steroids posttransplant; target sirolimus level 30 ng/mL in first 2 months following transplantation, then 15 ng/mL thereafter	Incidence of biopsy proven acute rejection 27.5% in sirolimus group vs. 18.4% in CyA group ($P = NS$); no significant difference in patient or graft survival; GFR was consistently higher in sirolimus-treated patients from month 2 onwards but not statistically significant at 1 year	Adverse side effects more frequently reported with sirolimus, including thrombocytopenia (45% vs. 8%), diarrhea (38% vs. 11%), and infectious pneumonia (15% vs. 5%), probably as a result of interaction with MMF
Kreis et al	*J Am Soc Nephrol* 15:809–817, 2004	480 on CyA/sirolimus/steroids randomized at 3 months to continue or to withdraw CyA; 36 months follow-up	Significantly better calculated GFR (59 vs. 47 mL/min) ($P < 0.001$) in the withdrawal arm with tendency to improved graft survival (85.1% vs. 91.2%) ($P = 0.052$)	More AR in withdrawal arm (5.6% v 10.2%) ($P = 0.11$)
Johnson et al	*Transplantation* 72:777–786, 2001	525 patients given CyA/sirolimus/steroids with sirolimus at 2 mg/day. At 3 months randomized to remain on sirolimus/CyA/steroids (215 patients) or undergo CyA withdrawal over 4 to 6 weeks continuing sirolimus/steroids with trough level of 20 to 30 ng/mL (215 patients)	At 12 months, creatinine (158 vs. 142 μmol/L (1.78 vs. 1.60 mg/dL) ($P < 0.01$) and GFR (57 vs. 63 mL/min) ($P < 0.01$) both better in patients undergoing CyA withdrawal; no significant difference in patient survival, graft survival, or incidence of biopsy-proven acute rejection	GFR progressively improved in sirolimus group throughout the 12-month study; no significant difference in new onset diabetes, hypercholesterolemia, or hypertriglyceridemia between groups
Oberbauer et al	*Transplantation* 76:364–370, 2003	Continuation of above study protocol with sirolimus trough concentrations of 15 to 25 ng/mL after 1 year; results at 2 years posttransplantation reported	Significantly lower creatinine in patients on sirolimus/steroids maintained at 2 years (128 μmol/L vs. 167 μmol/L (1.44 vs. 1.88 mg/dL) ($P < 0.001$)	Systolic blood pressure was also significantly lower in patients on sirolimus/steroid (141 vs. 134 mm Hg) ($P < 0.001$)
Flechner et al	*Transplantation* 74:1070–1076, 2002	61 subjects randomized to either sirolimus ($N = 31$), trough level 10 to 12 ng/mL, or CyA ($N = 30$), 200 to 250 ng/mL in addition to MMF/steroids; both groups induced with basiliximab	At 1 year sirolimus group significantly lowered creatinine (1.29 vs. 1.32 mg/dL (115 vs. 117 μmol/L) ($P = 0.008$) and creatinine clearances (77.8 and 81.1 mL/min^{-1}) ($P = 0.004$)	No significant difference in incidence of acute rejection; differences in renal function, although significant, are actually quite small
Flechner et al	*Am J Transplant* 4:1776–1785, 2004	2-year follow-up of the above study; 48 patients has biopsies and 55 had renal function studies	At 2 years sirolimus group significantly higher GFR (80.4 vs. 63.4 mL/min) and significantly more normal biopsies (66.6% vs. 20.8%)	Regression analysis of calculated GFRs from 1 to 36 months showed positive slope for sirolimus (3.36 mL/min/year) and negative slope for CyA (-1.58 mL/min/year) ($P = 0.008$)
Gonwa et al	*Transplantation* 74:1560–1567, 2002	Randomized to either full-dose CyA and 2 mg/day sirolimus ($N = 97$), or reduced dose CyA and concentration controlled sirolimus with trough levels of 10 to 20 ng/mL ($N = 100$); after 2 months, patients in the reduced-dose CyA/concentration controlled sirolimus group underwent CyA withdrawal over 4 to 6 weeks	No difference in patient survival, graft survival, or incidence of biopsy-proven acute rejection; patients in CyA elimination arm had significantly lower serum creatinine at 12 months (1.38 vs. 1.82 mg/dL (123 vs. 162 μmol/L) ($P < 0.001$)	No significant difference in incidence of clinically important infections, diabetes, hypercholesterolemia, or hypertriglyceridemia
Stegall et al	*Transplant Proc* 35 (Suppl 13A):125S–127S, 2003	85 patients randomized to tacrolimus/MMF/steroids ($N = 40$) or sirolimus/MMF/steroids ($N = 45$); both groups received ATG induction	No significant difference in GFR between groups at 4 months posttransplantation	At 4 months post transplantation, 8 of 45 patients discontinued sirolimus due to delayed wound healing ($N = 3$), acute rejection ($N = 2$), and severe hyperlipidemia ($N = 1$)
Ciancio	*Transplantation* 77:252–258, 2004	150 subjects randomized to sacrolimus with tacrolimus reduction, MMF with tacrolimus reduction or sirolimus with CyA reduction over the first postoperative year	No difference in graft and patient survival at 1 year; trend to worsening creatinine and low GFR for patient on CyA	1 year interim analysis; no difference in acute rejection episodes or infectious complications; less hyperlipidemia first two groups
Bumbea V	*Nephrol Dial Transplant* 2005, Jun 28 [Epub]	43 renal transplant recipients who switched from CNI to SRL for CAN or cutaneous malignancy	Improved graft function at 27 months	High discontinuation rate of 43%. One third of patients developed significant proteinuria. Not in print yet.

Abbreviations are: CyA, cyclosporine A; steroids, prednisolone/prednisone; MMF, mycophenolate mofetil; GFR, glomerular filtration rate; ATG, anti-thymocyte globulin.

discontinue CyA (with dose increase in sirolimus) or to continue on it. One-year graft (and patient) survival was similar with comparable acute rejection episodes, although a slight increase in acute rejection was observed at the time of CyA withdrawal. Patients on sirolimus had better GFRs than those maintained on CyA which was sustained over the 2-year follow-up. The Rapamune Maintenance Regimen Trial adopted a similar approach with stable allograft recipients on a triple immuno-suppression regimen of sirolimus/CyA/steroid random-ized at 3 months to continue or to withdraw CyA. At 36 months of follow-up, significantly better calculated GFR (59 versus 47 mL/min) was observed in the with-drawal arm together with a tendency to improved graft survival. Adverse effect profiles included predictably in-creased rate of hypertension, abnormal kidney function, edema, hyperuricemia, hyperkalemia, gingival hyperpla-sia, and *Herpes zoster* occurred significantly in the CyA continuation arm and abnormal liver function test results, hypokalemia, thrombocytopenia, and abnormal healing in the withdrawal arm [80]. Similar studies from Spain and Italy showed that a significantly lower rate of biopsy features of progressive tubular and interstitial chronic le-sions between basal and 1-year biopsies was evident on using this approach [81] and that fewer patients were di-agnosed with biopsy-proven CAN [82].

Since tacrolimus in combination with MMF is associated with fewer episodes of acute rejection than CyA [83], several studies have looked at the combination of tacrolimus and sirolimus. Ciancio et al [84] random-ized 150 first-transplant patients to receive tacrolimus and sirolimus, tacrolimus and MMF or Neoral and sirolimus. Each group received daclizumab at induction and steroids. Tacrolimus levels were tapered in the first group to 10, 8, and 6 ng/mL at 1, 6, and 12 months, re-spectively, and in the second group to 10 and 8 ng/mL at 1 month and 1 year, respectively. CyA levels were maintained at 225 and 175 ng/mL at 1 month and 1 year, while sirolimus levels remained unchanged at 8 ng/mL throughout. At 1 year, acute rejection was higher in the CyA group than in the others (14% versus 4% versus 4%; $P = 0.03$), although patient and graft survival were identical [77]. CyA-treated patients showed rising slopes of serum creatinine and concomitantly reducing creati-nine clearance at 1 year. Patients on sirolimus required more antihyperlipidemic medications than those on MMF [85].

Although these studies seem to indicate favorable out-comes for sirolimus in the short term, they do not ad-dress the problem of long-term allograft nephropathy, although the low incidence of acute rejection episodes, avoidance of CNIs and low 1-year serum creatinine [86] should improve long-term outlook. Unfortunately, sig-nificant selection bias exists as individuals recruited into these studies were of low immunogenic risk; hence, these results might not generalize to a more heterogeneous transplant population. Side-effect profiles appear to be a double-edged sword as amelioration of hypertension off CNIs is often at a cost of excessive hyperlipidemia on sirolimus, an important effect as cardiovascular mortality remains the number one cause of death. Nevertheless, the observations are encouraging and have formed the basis for further trials.

A small number of investigators have also studied the role of sirolimus in allowing dose reduction in CNIs once CAN has already developed. The trial of Saunders et al [87] recruited 31 patients with biopsy-proven CAN and randomized them to receive dose reduction in CyA (by 40%) with or without addition of sirolimus (2 mg/day). CyA trough levels were similar in the two groups but patients on sirolimus had a significant fall in 51 crea-tinine GFR (from 31.6 to 27.3 mL/min) at the end of the 6-month follow-up period, whereas controls did not. mRNA extraction from biopsy specimens showed that expression of TGF-β actually dropped in controls but re-mained constant on sirolimus while collagen expression actually increased in sirolimus-treated patients. These results are somewhat surprising given the well-known antiproliferative effects of sirolimus and should prompt further investigation using larger patient cohorts. More recently, Bumbea et al reported the findings of an in-vestigation of 43 renal transplant recipients who were switched from CNIs to sirolimus due to either CAN or recurrent cutaneous cancers. All patients were addition-ally on steroids together with either MMF or azathio-prine. Unfortunately, after 27 months of follow-up, only 59% of subjects were still on sirolimus. Intention-to-treat analysis demonstrated a sustained improvement in renal function at a cost of developing significant proteinuria in up to one third of subjects [88]. Although this is a small study, the high discontinuation rate is worrying.

Sirolimus, though of immense promise, has only re-cently been widely used, has very little experience in es-tablished CAN patients, and has a significant side-effect profile at least when used at conventional dose/blood level ranges. These can be idiosynchratic or dose-related effects such as edema, joint pains, skin rashes, mouth ulcers, pneumonitis, liver function disturbance, dys-lipidemia, thrombocytopenia, and thrombotic microan-giopathy [89, 90]. Nevertheless, sirolimus remains a useful adjunct immunosuppressant and the trials presented above have provided encouraging results for its use as primary immunosuppression in favor of CNIs or as an early switch a few months after transplantation. Its ex-act role in transplantation has yet to be established and will have to be the subject of further clinical trials with larger populations of more varied immunogenetics and immunologic risk. Further work on the use of sirolimus

once CAN has already developed should follow on the basis of the limited trial data already published.

CONCLUSION

CAN remains a very important and common cause of graft loss and a difficult clinical problem to tackle. Although many studies have been carried out in heterogeneous patient populations and involving addition or manipulation of a number of different agents, in general, subjects have been carefully selected and small in number. So this is quite unlike "clinical practice." More attention needs to be paid to which histologic markers at allograft biopsy best predict a favorable outcome following on from manipulation of immunosuppression. Additionally, there is significant interobserved variation in the classification of CAN according to the Banff criteria [91]. Making conducting clinical research a more difficult task because different degrees of fibrosis may respond diversely to manipulation of immunosuppression.

Furthermore, nearly all trials present short-term data, with only a few reporting long-term findings, using plasma creatinine or GFR as a surrogate marker for long-term survival prospects. One would indeed hope that short-term improvement will translate to long-term gain but this is by no means a certainty. Firm conclusions on trials with these limitations are therefore difficult to make. Certainly, if the pathologic process seems intimately related to the use of calcineurin inhibitors, it would appear logical to switch from CNI to non-CNI–based protocols at least once CAN has developed especially since trial data on switching from CyA to tacrolimus are limited and still unconvincing. A corollary of this philosophy is the use of protocol implantation followed by allograft biopsies at 3 to 6 months postengraftment to detect increasing fibrosis or subclinical CAN (rather than waiting for progressive allograft dysfunction) on the basis of which drug therapy can be altered as above. Alternatively, one could try to avoid CNI exposure from the outset. Though this sounds reasonable, it too should be subjected to the rigor of a randomized controlled trial.

Azathioprine carries an excess risk of acute rejection during a switch from CyA but seems on the basis of limited data to ameliorate CyA toxicity and to improve lipid profiles and blood pressure control by allowing dose reduction or withdrawal of CNI. However, the studies using azathioprine are quite limited and therefore its effects on long-term graft survival, development, and progression of CAN cannot be firmly stated.

MMF has had perhaps the most significant amount of investigation, with most papers demonstrating an improvement in graft function at least in the short term without precipitating rejection episodes, although publication bias may be playing a role here. Unfortunately, there are very few long-term data and most trials have used relatively large doses of MMF (of the order of 2 g/day), thereby reporting high incidences of adverse effects. Given the interactions between MMF and CNIs, we would suggest monitoring of MPA levels in future trials in order to individualize MMF doses for patients. The evidence that the use of MMF is, per se important (as opposed to its nonspecific permissive role in allowing a reduction in CNI exposure) needs confirmation with more precisely designed trials addressing this point. Clearly in 2006 many transplantation centers now routinely use MMF therapy at induction, so in this case simple CNI reduction or withdrawal should be relatively straightforward to achieve.

Investigations into sirolimus have so far been relatively encouraging, with demonstration of its efficacy in the short term to substitute for CNIs or to allow lower doses to be used without an increase in acute rejection episodes. However, there are limited long term results on using sirolimus in lieu of calcineurin inhibitors once CAN has developed and therefore this approach cannot yet be strongly recommended. The side-effect profile of this drug at conventional doses is a cause for concern.

Using the three criteria we outlined in the introduction—the success of the intervention at arresting renal function decline, the side-effect profile of any new immunosuppressive agents added (including acute infectious complications as a result of changes in the overall potency of immunosuppression), and the rate of acute or chronic rejection (and subsequent graft loss), we feel that the present evidence best favors the reduction/elimination of CNI under MMF cover. We must enter the caveat, however, that much more clinical information and trial evidence is required before the definitive approach to the prevention or treatment of CAN can be recommended. These concerns are in the process of being addressed with a series of ongoing switch studies aimed at answering the question of whether switching from or reducing CNI combined with introduction of MMF, sirolimus, or everolimus may save renal transplant function or reduce the progression of CAN. These trials include the ongoing Trancept and Intercept Studies (MMF, ongoing), the EliTE-symphonie study (MMF, ongoing), the 316 sirolimus study (sirolimus, ongoing), and the ASCERTAIN Trial (everolimus, recruiting). In the meantime, while awaiting the completion of these trials, it would appear reasonable to reduce doses of CNIs once CAN has developed and to substitute another agent to prevent acute rejection. MMF, given that it has the largest share of clinical data thus far would seem to be an appropriate first choice agent.

Finally, important though the impact of altering immunosuppression may be, it must be remembered that there are many shared non-immunologic risk factors between CAN and cardiovascular disease, which remains the most important cause of death and graft loss. It is

important therefore to try and control these as well, in particular anemia, hypertension, proteinuria, and hyperlipidemia, and the importance of control of blood pressure [92] as well as adjunctive therapies such as angiotensin-converting enzyme (ACE) inhibitors [93] and statins [9] cannot be overstated. There are differences between immunosuppressive compounds regarding risk factors for cardiovascular disease and it is perhaps another argument for the metabolically neutral MMF over the cholesterol-raising sirolimus and the prohypertensive, diabetogenic, and prodyslipidemic CNI. However, one must note that there is no evidence that such differences really translate into differences in real cardiovascular events.

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