Safety and Efficacy of a Calcineurin Inhibitor Avoidance Regimen in Pediatric Renal Transplantation

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Thirty-four children were entered into a pilot trial of calcineurin inhibitor avoidance after living-donor kidney transplantation, the CN-01 study. Patients were treated with anti-CD25 mAb, prednisone, mycophenolate mofetil, and sirolimus. Twenty patients were maintained on the protocol for up to 3 yr of follow-up. One enrolled patient did not receive the transplant because of a donor problem, eight terminated because of one or more rejection episodes, four terminated because of adverse events, and one was lost to follow-up. Two grafts were lost, one as a result of chronic rejection and the other as a result of posttransplantation lymphoproliferative disorder. There were no deaths. The 6- and 12-mo acute rejection rates were 21.8 and 31.5%, respectively. GFR were stable throughout the course of the study, with a slight downward trend by 6 mo after transplantation followed by a slight upward trend to a mean of 70 ml/min thereafter. Early surveillance graft biopsies frequently showed focal interstitial mononuclear cellular infiltrates without overt vasculitis or tubulitis, but these infiltrates disappeared without treatment. Anti-HLA class I and II antibodies were detected in three patients before transplantation, and all three had acute rejections, including the two patients who lost their grafts. *De novo* anti-HLA Ab production occurred in only one patient after transplantation. There were two episodes of Epstein Barr virus–related posttransplantation lymphoproliferative disorder, one of which developed after the patient had been terminated from the study. It is concluded that calcineurin inhibitor–free immunosuppression can be safe and effective in pediatric living-donor renal transplantation. However, further modifications that are designed to lessen early rejection rates and decrease complications should be tested before this approach is used routinely.

J Am Soc Nephrol 17: 1735–1745, 2006. doi: 10.1681/ASN.2006010049

Renal transplantation has long been considered the preferred treatment of ESRD in children (1–3). For years, however, children had poor outcomes and were considered to be high risk as compared with adults (4). Improvements in donor selection, surgical techniques, and knowledge of immunosuppressive drug doses and metabolism in children have led to substantial improvements in pediatric kidney graft and patient survival (5,6). The improvement in outcomes in children have exceeded those of adults, and children who are younger than 10 yr now have the best outcomes of all age groups of kidney transplant recipients (6).

Much of the improvement in organ transplantation outcomes has been due to the prevention of early acute rejection episodes and prompt identification and treatment when they occur (5,7–9). Whereas some single-center reports have described rates of acute rejection episodes as low as 13 to 26% at 1 yr in selected groups of pediatric recipients (10–13), large multicenter studies have reported rates as high as 27 to 59% (14–19). Registry studies have reported that overall 6-mo acute rejections rates of 45% in deceased-donor and 32% in living-donor kidney transplants in 1992 fell to 21 and 20%, respectively, by 2003 (20). The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) reported a 57% first-year acute rejection rate in pediatric renal transplant recipients in 1987 that decreased to 32% by 2001 (5).

One of the major reasons for decreased acute rejection rates and improved short-term outcomes is the improvement in immunosuppressive medications (21,22). Among these, the calcineurin inhibitors (CNI) are the most potent and likely the most important in improving outcomes (23). However, these medications have multiple adverse effects, among which the most significant is nephrotoxicity (24–27). Chronic CNI-associated nephrotoxicity is thought to be one of the antigen-independent factors related to the progression of chronic allograft nephropathy (CAN). Protocol biopsies of pediatric kidney transplant recipients have demonstrated interstitial fibrosis and tubular atrophy characteristics of CNI nephrotoxicity (28,29). Importantly, as many as 15% of extrarenal transplant recipients

Received January 16, 2006. Accepted March 25, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1706-1735

develop chronic renal insufficiency, and CNI toxicity is thought to play a major role in the development of that disorder (30). In addition, CNI have been associated with hypertension, increased rates of steroid-associated diabetes, and neurologic complications (31).

There is very little experience with the use of the target of rapamycin (TOR) inhibitors sirolimus and everolimus in pediatric organ transplantation (32–36). This class of immunosuppressants has a novel mechanism of action that is different from all other antirejection medications that are used for transplantation (37,38). The major complications of the TOR inhibitors include hyperlipidemia, thrombocytopenia, and poor wound healing (38). Sirolimus has been used in adult kidney transplant recipients in CNI avoidance or withdrawal studies (39). These studies generally have demonstrated better long-term GFR in recipients in whom there is CNI avoidance or withdrawal as compared with those who receive chronic CNI-based immunosuppression. However, acute rejection rates may be somewhat higher. Because of these early results in adult renal transplant trials, we undertook a pilot study of CNI avoidance in pediatric renal transplantation.

Materials and Methods

Study Design and Patient Enrollment

The Cooperative Clinical Trials in Pediatric Transplantation (CCTPT) is a cooperative research program sponsored by the National Institute of Allergy and Infectious Diseases. Four participating centers of CCTPT entered patients into this study, identified as CN-01 study, which was designed as a single-arm pilot study in which all patients received daclizumab induction, prednisone, mycophenolate mofetil (MMF), and sirolimus. The primary objective of the study was to determine whether the rejection risk was low enough in the first year after transplantation to permit the use of chronic CNI-free immunosuppression in pediatric transplantation. Adverse events, surveillance graft biopsies, and changes in measured GFR were monitored carefully. Living-donor kidney transplant recipients who were younger than 21 yr and receiving their first or second graft were eligible. The study protocol was approved by the institutional review board at each study site as well as by the National Institute of Allergy and Infectious Diseases. Informed consent was obtained from the patients who were 18 yr and older and from the parents of patients who were younger than 18 yr before their enrollment in the study. Patients who were older than 12 yr also provided assent.

Immunosuppression

All patients received a loading dose of sirolimus 1 d before transplantation. They received the anti-CD25 mAb daclizumab intraoperatively and every 2 wk up to 8 wk after transplantation. Maintenance immunosuppression consisted of prednisone, which was begun at 2 mg/kg per d and tapered to 0.15 mg/kg per alternate day; MMF at a dose of 1200 mg/m^2 per d, divided twice daily; and sirolimus, which was administered on a twice-daily schedule and at a dose that was designed to maintain whole-blood trough levels of 20 to 25 ng/ml for the first 2 mo, 20 ng/ml for months 3 to 6, and 15 ng/ml thereafter. Patients were followed for 3 yr.

Graft Evaluation

GFR were measured by elimination of radiolabeled technetium at 3, 6, 12, 24, and 36 mo after transplantation. Surveillance kidney transplant biopsies were obtained at the time of the transplant procedure (postperfusion) and at 3, 6, and 12 mo after transplantation. Two cores were obtained, one of which was used for routine processing and review at the clinical center. The other core was split into two pieces, and both were snap-frozen in liquid nitrogen and stored for later analysis. One piece was used for mRNA analysis, and the other was used for immunohistologic analysis.

Alloantibody Detection

Blood samples for alloantibody detection were obtained before transplantation; at 3, 6, and 12 mo after transplantation; and at the time of suspected rejection. A flow cytometric Luminex XY platform was used to detect class I and class II alloantibody (OneLambda, Inc., Canoga Park, CA). Microbeads are coated with purified class I and class II HLA antigen that represents all common and many rare antigens. Test serum was maintained at −80°C until just before testing. Serum samples were centrifuged at 8000 rpm for 5 min to remove aggregates and tested undiluted. A total of 20 μl of test serum, as well as positive and negative control sera, was incubated with 5 μl of LABScreen class I and class II beads. One microliter of 100× conjugated anti-human IgG per test sample was diluted in 99 μl of wash buffer and incubated with the beads for an additional 30 min. All samples were analyzed within 1 h of completion. The LABScan analyzer with LABScreen analysis software was used for data acquisition. Serum reactivity was assessed by the PE fluorescence shift for each HLA-coated bead after correction for nonspecific binding to the negative control bead. Reactions are graded as positive, negative, or gray zone depending on the reactivity of the sample serum in comparison with the control serum.

Statistical Analyses

Statistical analysis was designed to determine whether the acute rejection rate was acceptable to permit subsequent studies. We proposed that a rejection rate in the first 6 mo of 40% would be unacceptable and that a rate <20% would be desirable, especially if the patients were free of CNI adverse effects. Therefore, if there were sufficient evidence to conclude that the acute rejection rate was <20 or >40%, then the study would have been terminated. The sequential testing procedure used was a truncated extension of the sequential probability ratio test. With the study sample size of 35, the design type I and type II error rates were 8 and 12%, respectively. The time course of repeated lipid and GFR measures was analyzed using generalized linear models with parameters estimated using generalized estimating equations.

Results

Enrollment and Primary Outcome

Thirty-four patients were enrolled between February 2001 and August 2003. One enrolled patient did not receive the transplant because of a problem uncovered in the donor. Six patients had at least 3 yr of follow-up, and 91% had at least 1 yr of follow-up; the mean follow-up was 2.1 yr. Of those 33, nine were younger than 6 yr, four were 6 to 12, and 20 were older than 12. Twenty-one were boys, and there were no black patients. Ten patients received preemptive transplants, two had previously received a kidney transplant, and three donors were unrelated. There were no episodes of delayed graft function. Patients received sirolimus on a twice-daily schedule (35), and target levels were achieved by the second week and maintained for the rest of the study (Figure 1).

Eleven patients had 14 acute rejection episodes, seven within the first 6 mo and four after the first 6 mo. The plot of actual

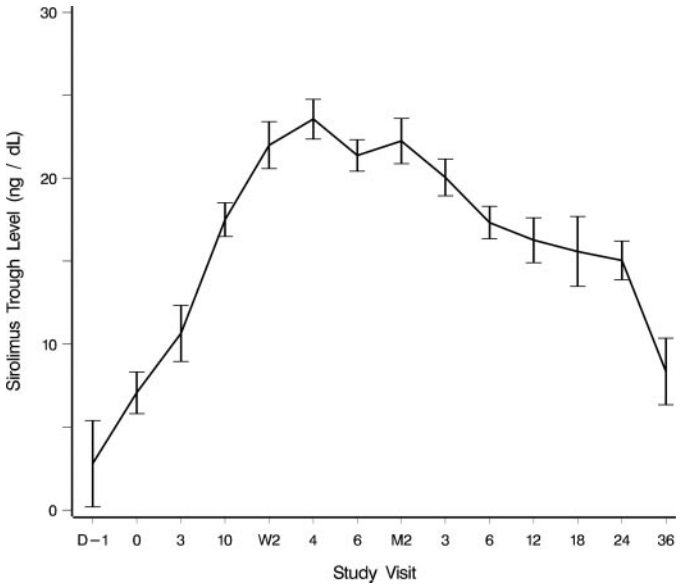

Figure 1. Mean and SE sirolimus trough levels from the time of transplantation until 36 mo after transplantation in children in CN-01 study.

acute rejection rate in the first 6 mo *versus* sequential probability ratio test boundaries is shown in Figure 2. The test statistic did not approach the upper bound and remained close to the lower bound throughout the study. Two grafts were lost, one at 94 d as a result of recurrent rejection and posttransplantation lymphoproliferative disorder (PTLD) and the other at 732 d as a result of chronic rejection. There were no deaths. Thirteen (39%) patients withdrew from the study treatment protocol (Table 1). Of these, eight were withdrawn because of one or more rejection episodes, four were withdrawn because of adverse events or complications, and one was lost to follow-up. Of those with rejection, two lost their grafts and six still have functioning grafts after treatment of rejection. In five of these cases, maintenance immunosuppression was changed by discontinuing MMF and replacement with tacrolimus. In one case, the patient was left on the study protocol of sirolimus, MMF, and steroids; that patient has not had any more episodes of acute rejection. Of the four patient who were withdrawn because of adverse events, two had neutropenia and one had diarrhea and vomiting; all three of these had resolution of symptoms when tacrolimus was substituted for MMF. One patient had a lymphocele and poor wound healing that necessitated the temporary discontinuation of sirolimus; he is currently receiving sirolimus, tacrolimus, and prednisone.

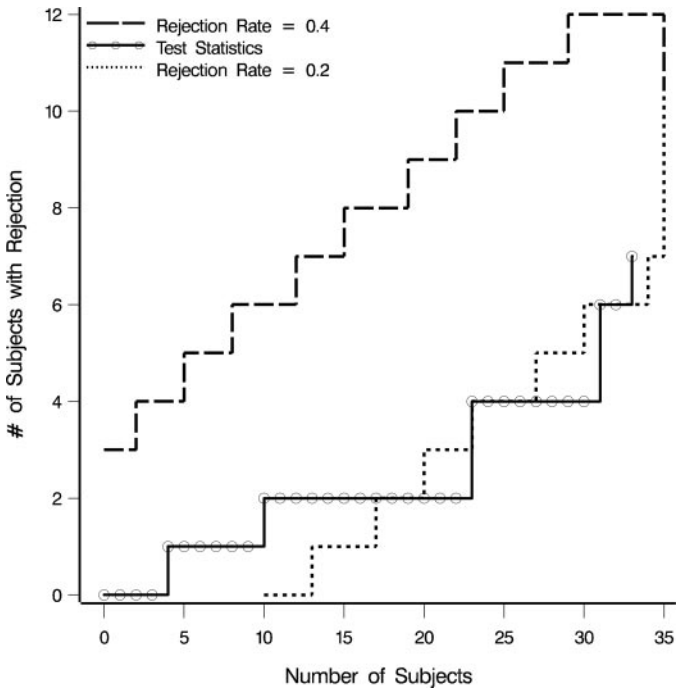

Figure 2. Stopping guideline as determined by sequential probability ratio test. The upper dotted line indicates a rejection rate inconsistent with the lower 20% bound in the first 6 mo after transplantation, the lower dotted line indicates a rejection rate inconsistent with the 40% bound, and the solid line indicates the rate in CN-01 study.

Acute Rejections

As noted above, 11 patients experienced 14 episodes of biopsy-proven acute rejection. The incidence of acute rejection is shown in Figure 3. The 6-mo and 1-yr rates determined by Kaplan Meier estimates were 21.8 and 31.5%, respectively. Three of these episodes were identified in surveillance biopsies; 11 were classified as acute cellular, one as acute cellular/vascular, and one as acute and chronic rejection. In 12 cases of rejection, the serum creatinine was <2.0 mg/dl at the time of diagnosis. One of the two instances of rejection that were diagnosed with a creatinine >2.0 mg/dl was in the patient with chronic rejection. Both patients with previous transplants had pretransplantation alloantibodies, and both had acute rejection episodes. All rejection episodes were treated with Solu-Medrol pulses, and five also received Thymoglobulin for persistent ($n = 4$) or concomitant ($n = 1$) vascular rejection. All episodes responded to the treatment and resulted in stabilized or lowered creatinine concentrations except for the patient who had chronic rejection, who went on to lose her graft 2 mo later.

Complications

A total of 305 adverse events were reported, 61% of which were classified as either not related or remotely related to the test therapy (Table 2). A total of 142 adverse events were classified as moderate severity, 68 of which were possibly, probably, or definitely related to the treatment; and 34 severe adverse events, 20 of which were possibly or probably related to the therapy. Infections, vascular disorders, and gastrointestinal disorders were the most frequently reported adverse events. Four patients had neutropenia or anemia that was classified as serious, and two withdrew from study therapy because of it. Thirteen other reports of neutropenia or anemia were classified as mild. Mean hematocrit and white blood cell and platelet counts are shown in Figure 4. Six lymphoceles reported, four of which were listed as serious. One episode of poor wound healing led to discontinuation of sirolimus. There

Table 1. Reasons for withdrawal from study

Center	Transplant Date	Termination Day	Reason for Early Termination	Early Termination Explanation	Reason for Therapy Termination
1	2/13/2001	177	Investigator/study decision	Started on tacrolimus 8/14/01	Rejection
1	4/28/2003	199	Other	Second rejection	Rejection
1	9/25/2001	659	Other	Second rejection	Rejection
91	7/10/2003	39	Investigator/study decision	Acute rejection	Rejection
91	5/21/2003	195	Investigator/study decision	Borderline acute rejection	Rejection
91	7/25/2001	111	Investigator/study decision	Acute rejection on study	Insufficient therapeutic response
91	10/17/2001	104	Investigator/study decision	Neutropenia	Adverse event
91	9/4/2002	158	Other	Parents requested termination	Adverse event
91	11/13/2002	99	Investigator/study decision	Acute rejection	Rejection
91	7/16/2003	36	Investigator/study decision	Complication of delayed wound healing	Adverse event
91	5/14/2003	69	Investigator/study decision	Concern of acute rejection	Rejection
91	10/16/2002	135	Withdrawn	Adverse event of neutropenia	Adverse event
91	10/30/2002	741	Investigator/study decision	Patient lost to follow-up/unable to reach	Lost to follow-up

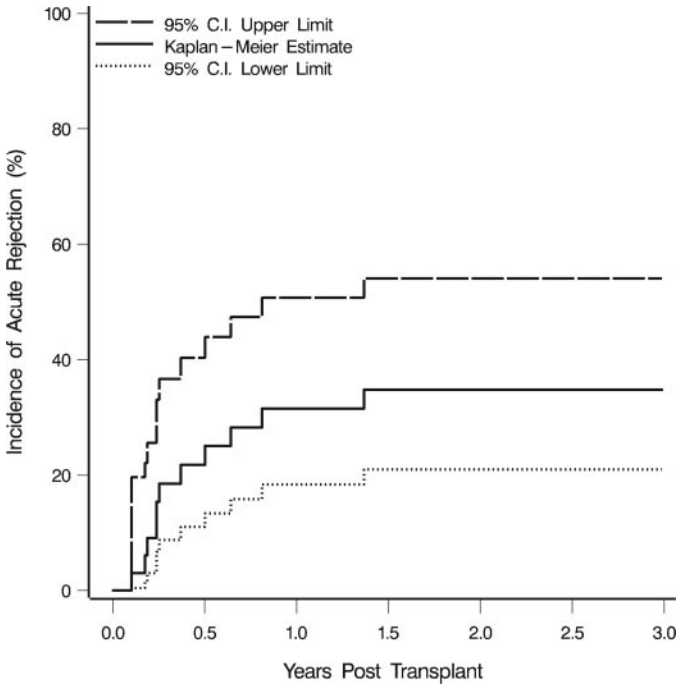

Figure 3. The incidence of acute rejection up to 3 yr after transplantation in the patients in CN-01 study. The dotted lines indicate the 95% confidence intervals (CI).

were four episodes of mouth ulcers, one of which was classified as serious. At 1 yr, 45% of patients were receiving antihypertensive medications. Of the 215 assessments for proteinuria, 31 samples that were obtained after 1 mo posttransplantation had protein/creatinine ratios >0.5. Eight patients had repeated ratios >0.5, four of whom had intermittently raised ratios and four of whom had sustained elevated ratios. Of the four with intermittent ratios >0.5, two had elevations concurrent with rejection episodes, one had delayed graft function followed by a urinoma and repeated urinary tract infections, and one had levels that fluctuated from a high of 0.82 to a low of 0.18, with a trend toward lower levels at later time points. Of the four with persistently elevated levels, two had early acute rejection episodes, one had proteinuria before transplantation that persisted after transplantation, and one had donor pathology observed in the implantation biopsy that worsened somewhat on the surveillance biopsies.

Twenty-one (64%) patients had infections; the most common infection was a urinary tract infection. There was one report of esophageal candidiasis, one episode of pulmonary aspergillosis, and one episode of *Pneumocystis carinii* pneumonia, all of which were treated successfully. One episode of cytomegalovirus viremia was detected but no clinical cytomegalovirus disease. There were two episodes of Epstein-Barr virus (EBV)-related PTLD, both in EBV-Ab–negative recipients of grafts from EBV-Ab–positive donors. One of these two patients was a 5-yr-old girl who had no rejection episodes and presented with

Table 2. Categories of adverse events

Severity	Relationship to Sirolimus						
	Unrecorded	Not	Remote	Possible	Probable	Definite	Total
Mild	1	86	20	14	7	0	128
Moderate	7	57	10	49	17	2	142
Severe	2	11	1	11	9	0	34
Life-threatening	0	0	0	0	1	0	1
Total	10	154	31	74	34	2	305

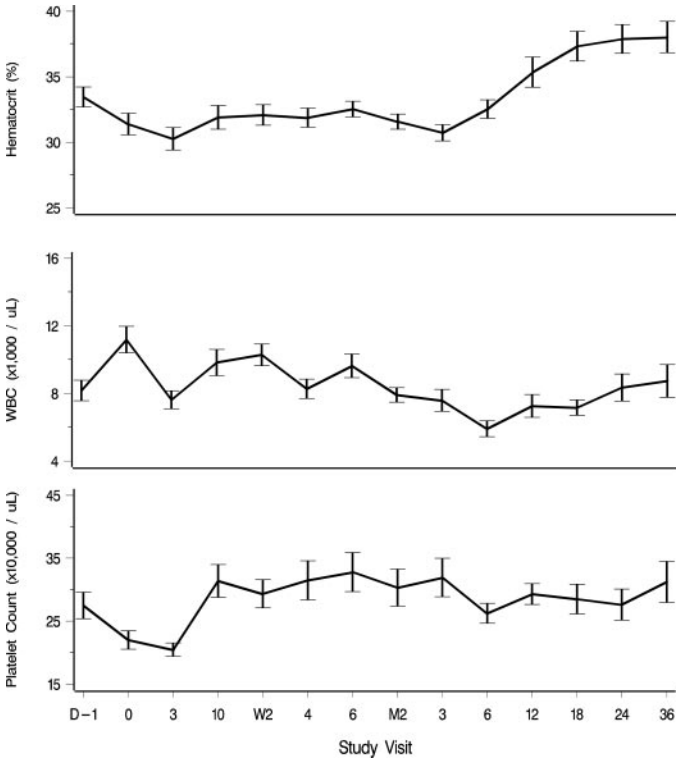

Figure 4. Mean hematocrit and white blood cell (WBC) and platelet counts in patients in CN-01 study. The bars indicate the SD of the mean.

asymptomatic papilledema 10 mo after transplantation. She was found to have multiple intracranial lesions on head magnetic resonance imaging, and brain biopsy identified PTLD. Her MMF was discontinued, and her sirolimus dose was lowered. Her prednisone dose was increased to 20 mg/d because of the ocular nerve pressure. She received high-dose rituximab treatments twice weekly for 4 wk, and the lesions regressed. A repeat magnetic resonance imaging scan 4 mo later showed worsening of one lesion, so she received a second round of rituximab treatment. All lesions have resolved, and her vision has improved. She currently is treated with sirolimus and prednisone, which is being tapered to an alternate-day schedule. Her serum creatinine is 0.7 mg/dl. The other patient with PTLD was a 17-yr-old boy who had acute rejection on day 39 and was withdrawn from the study at that time after he had been treated with Thymoglobulin and Solu-Medrol. His maintenance treatment was changed to tacrolimus, sirolimus, and prednisone, and he seemed to respond to that treatment. One month later, a repeat graft biopsy demonstrated PTLD in the graft, and his immunosuppression was reduced. He was treated with rituximab, but his renal function deteriorated and he experienced a splenic hemorrhage. He underwent splenectomy and transplant nephrectomy 3 mo after transplantation.

Lipid levels generally were elevated in all patients. For example, cholesterol rose rapidly after posttransplantation day 3, peaked at 74 mg/dl above baseline ($P < 0.001$) at week 2, and subsequently declined. After month 2, the elevation relative to baseline ranged from 16 to 30 mg/dl with P values ranging from 0.05 to 0.10. Mean triglycerides and total, HDL, and LDL cholesterol levels are shown in Figure 5. A total of 79% of patients received lipid-lowering medications.

GFR

GFR was measured by elimination of radiolabeled technetium. Mean gross GFR is shown in Figure 6; there was a slight decline in mean GFR during the first 6 mo, from 86 to 60 ml/min, but a moderate increase during the subsequent 30 mo and was 70 ml/min at 36 mo. Terminal GFR values were 16.2 ml/min lower than at month 1 ($P = 0.12$) and 9.4 ml/min higher ($P = 0.34$) than at the observed 6-mo minimum. Although normalized GFR declined during the first 6 mo, no further decrease was observed during the next 2.5 yr. Mean Δ GFR for the same patients showed the same pattern, indicating that improvement in GFR was not attributable to loss of study patients with poor renal function or graft loss. GFR corrected for body surface area decreased slightly during that time span because most of the children were growing.

Biopsy Specimens

In addition to an intraoperative biopsy, surveillance protocol biopsies were collected at 3, 6, and 12 mo after transplant. More than 70% of protocol biopsies at 3 mo showed one or two small foci of interstitial mononuclear cells (Figure 7, a and b). These cells typically showed no infiltration across the basement membrane or were associated with only mild tubulitis (Banff t1, with no more than four inflammatory cells in the most inflamed tubule) and negligible interstitial (Banff i), vascular (Banff v0), or glomerular (Banff g0) inflammation, leading to classification in the "borderline" category (40). Evaluation of subsequent protocol renal biopsies at 6 and 12 mo commonly showed resolution of these infiltrates (Figure 7, c and d). These latter

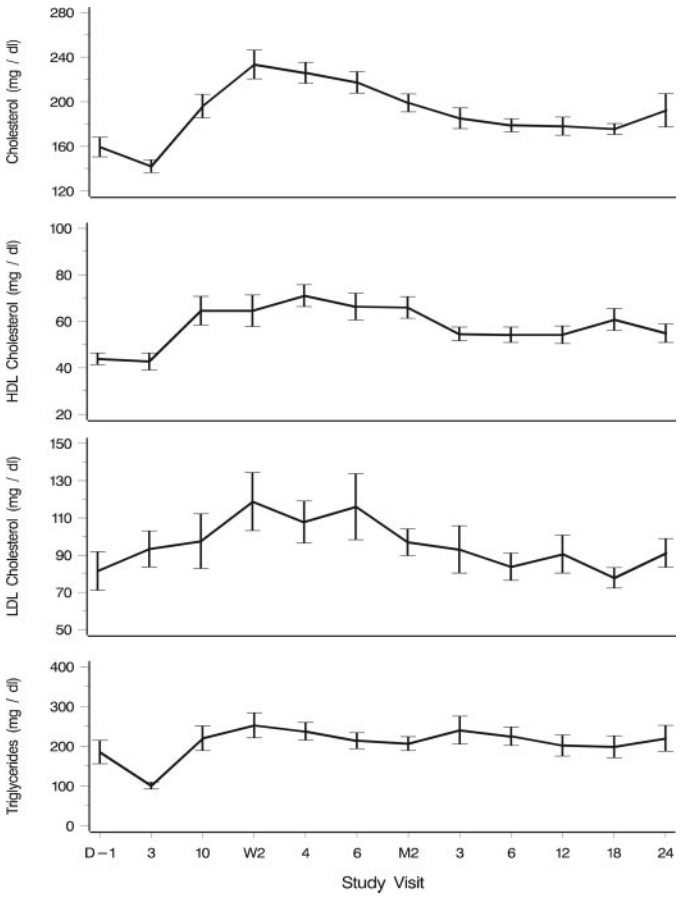

Figure 5. Mean total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides in patients in CN-01 study. The bars indicate the SD of the mean from the repeated measurements model.

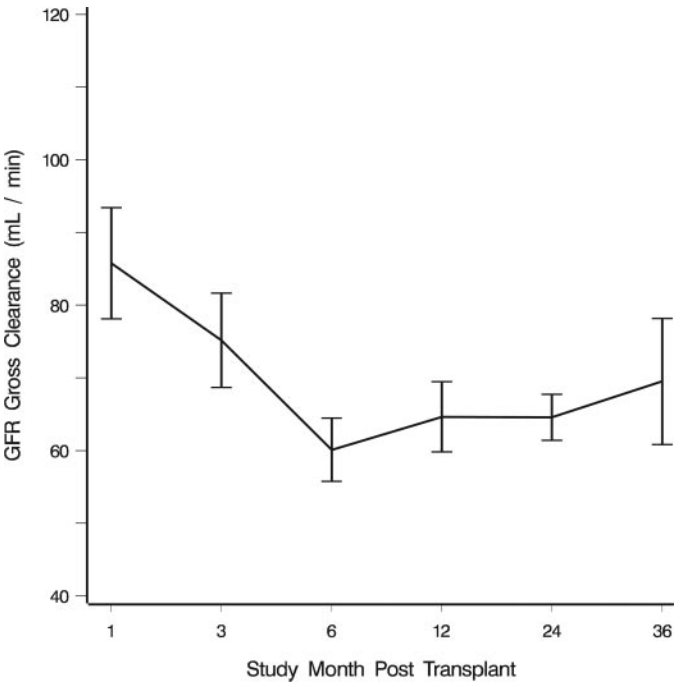

Figure 6. Mean gross GFR up to 36 mo after transplantation of patients in CN-01 study. The bars indicate the SEM from the repeated measurements model.

specimens generally were free of interstitial fibrosis and tubular atrophy. In contrast, 11 of the 14 biopsies that were obtained during acute rejection showed acute cellular rejection with extensive interstitial inflammation (Banff i2) and moderate or severe tubulitis (Banff t2 or t3; Figure 7, e and f). Of the remaining three cases, two showed evidence of both acute and chronic (Banff ci2, ct1, cv1, and cg0) rejection, and one showed acute cellular and vascular rejection (Banff i2, t2, v2, and g0).

Alloantibody Production

A total of 101 serum samples in 33 patients were analyzed for HLA-Ab. Before transplantation, five (15%) of 33 patients had HLA Ab detected (Table 3). Three had both class I and class II antibodies; all three had acute rejection episodes, and two of them lost their grafts. Of 27 patients with posttransplantation serum samples available, five had HLA Ab detectable at some point in the first posttransplantation year, and four had had pretransplantation HLA antibodies. Only one of 27 developed new HLA Ab after transplantation, and upon further confirmatory testing, plasma renin activity was <1% for both class I and class II and no specificities were identifiable, suggesting insignificant Ab levels or false positivity of the screening assay.

Discussion

The results of this study support the concept that kidney transplantation in children can be performed safely and effectively without the use of CNI for chronic immunosuppression. The majority (60%) of the patients tolerated the combination of sirolimus, MMF, and alternate-day prednisone without serious complications and had excellent long-term graft function, without deterioration of GFR after 6 mo. In general, the 1-yr graft biopsies of theses patients had very little evidence of interstitial fibrosis or other signs of CAN. Although we did not have a control group for comparison, long-term kidney biopsies of patients who receive CNI typically have signs of CAN by that time (28,29,41), which has been used as one of the reasons for attempting CNI avoidance protocols (42,43).

The doses of anti-CD 25 Ab, MMF, and prednisone were typical doses that were used for pediatric kidney transplantation at the time. The target levels of sirolimus that were used in this study were based on early studies of CNI-free protocols in adults (44). Earlier, uncontrolled use of sirolimus in children suggested that they may have an increased rate of metabolism of the drug, requiring more frequent dosing. This observation was confirmed in our study, with a very short half-life of sirolimus of 12 to 18 h (35). Therefore, the use of twice-daily dosing did seem appropriate. The doses that were required to attain these target levels with this dosing schedule frequently were higher than doses that are given to adults who are on CNI-based protocols. Despite the high doses and levels of sirolimus, the expected adverse effects did not limit the use of the drug. One patient discontinued the use of sirolimus because of problems with wound healing; two patients had neutropenia

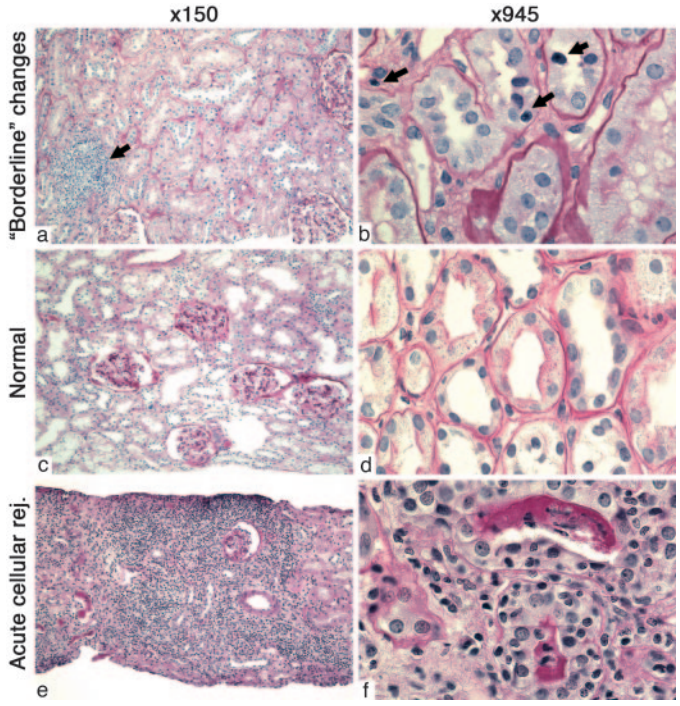

Figure 7. Representative biopsies of patients in the CN-01 study. (a and b) Three-month surveillance biopsies that demonstrated small foci of interstitial mononuclear cells classified as "borderline" and generally were not interpreted as representing acute rejection (rej). Resolution of these infiltrates was noted in 6- and 12-mo surveillance biopsies (c and d). These later specimens generally were free of interstitial fibrosis and tubular atrophy. In contrast, specimens obtained from patients with clinical acute rejection demonstrated typical interstitial inflammation and tubulitis (e and f).

that resolved with discontinuation of MMF while continuing the sirolimus. Otherwise, peripheral blood cell lines generally were well maintained. Most patients did have hyperlipidemia, but it was controlled with lipid-lowering medications. Sustained proteinuria beyond 1 mo was identified in four patients, one of whom had donor pathology identified at the implantation biopsy, and two others had early acute rejection episodes. The 45% rate of treated hypertension at 1 yr was substantially lower than the 70% rate generally reported in pediatric renal transplant recipients (5).

It should be noted that this study was designed to include typically low-risk living-donor pediatric kidney transplant recipients, and it may not be appropriate to extrapolate these results to higher risk groups. Indeed, minimization protocols may not be appropriate for sensitized or high-risk transplant recipients. There were no black patients enrolled in this study, which was regrettable but not deliberate. Also, the percentage of preemptive transplants was slightly higher than usual pediatric rates. The rate of acute rejection episodes was higher than desired, but it did stay within the predetermined safety limits (Figures 2 and 3). Ideally, a rate of $<20\%$ in the first year would have been considered an excellent outcome and would have supported an early termination of the study because of better-than-expected outcome. However, the 1-yr rate of 31.5% is comparable to other reported pediatric kidney transplant reports and comparable to CNI-free protocol results in adults (5,44). Single-center pediatric transplant outcome reports are difficult to assess because of small sample size and concern about patient selection criteria. Reports from larger multicenter trials and from registries generally have cited higher acute rejection rates, from 27 to 59% (5,14–19). Two large controlled trials of CNI avoidance in adult kidney transplantation showed a 35% acute rejection rate in the sirolimus plus azathioprine/MMF group *versus* a 29% rate in the control immunosuppression of cyclosporine and azathioprine/MMF (39). One controlled trial in adults resulted in low 1-yr rejection rates for both the sirolimus (6.4%) and cyclosporine (16.6%) groups, but these results have not been duplicated in other studies (45). In all of these trials, long-term GFR was higher and better maintained in the CNI-free group than in those who were treated with CNI. In one controlled trial, 31 adults who were treated with sirolimus/MMF/steroids were compared with 30 who were treated with cyclosporine/MMF/steroids; at 2 yr, the sirolimus group had higher measured GFR (61 *versus* 49 ml/min) and substantially reduced incidence of CAN (46). This pattern was seen in this trial, with a very stable GFR of 70 ml/min at 3 yr after transplantation; in contrast, the typical pattern for kidney transplant recipients who are treated with CNI is to have an inexorable and continuing decline in GFR after transplantation.

Surveillance biopsies frequently demonstrated a substantial cellular infiltrate that was particularly concerning in the early phases of the study because these infiltrates might have been a sign of early or subclinical rejection. However, these focal infiltrates were not associated with vasculitis or significant tubulitis, and the biopsies were not interpreted as indicating rejection, especially because they were not associated with evidence of graft dysfunction and they seemed to resolve spontaneously. Examination of protocol renal transplant biopsies has previously shown significant interstitial inflammation and/or mild tubulitis in the absence of graft dysfunction, leading to the de-emphasis of such features as diagnostic criteria for rejection and their depiction as borderline changes or borderline rejection (40). The literature indicates that borderline changes may or may not resolve with increased immunosuppression, and their prognostic significance may vary according to the immunosuppressive protocol, pediatric *versus* adult recipient population under study, and other factors (47–51). Although mRNA and immunohistologic characteristics of these infiltrates will be reported separately, it is clear from this and other studies that protocol biopsies can be used safely to monitor renal transplant recipients (52,53). Moreover, such biopsies suggest that at least in some patients, an early and transient posttransplantation inflammatory or immune response is under way. Whether this response in CNI-free renal transplant recipients has similarities to early intragraft events that are detected experimentally and termed as acceptance reactions remains to be determined (54). Importantly, early infiltrates seemed to resolve spontaneously, and later surveillance biopsies were free of interstitial fibrosis and tubular atrophy (Figure 7, c and d).

Limited studies have examined the prevalence and natural

Table 3. Summary of HLA antibody status throughout study[a]

Patient	Class I			Class II			Comments
	Screen[b]	PRA	Specificity	Screen[b]	PRA	Specificity	
Patients with class I and class II HLA-Ab detected before transplantation ($n = 3$)							
1							
0	1		IS	0.5		IS	Despite insufficient sample available for testing of PRA and specificity, class II Ab were no longer detectable and class I Ab were reduced at 3 mo after transplantation.
3 mo	0.5		IS	0		IS	
2							
0	1	35%	A2, A68	1		DQ2	Class I Ab were reduced during the first 12 mo compared with previous transplant; class II Ab remained relatively unchanged.
3 mo	0.5	16%	A2	1		DQ2	
6 mo	0.5	3%	A2	1		DQ2	
12 mo	0	4%	B46	1		DQ2	
other (1)	1	25%	A2	1		DQ2	
other (2)	0.5	11%	A2	1		DQ2	
3							
0	1	0%	None called	1		DR4	Despite a positive class I screening test at time 0, no class I PRA or specificities were defined; class I Ab was transiently detectable at 3 mo; class II PRA continued to increase up to 6 mo after transplantation.
3 mo	1	36%	Cw9, B35, B18, B51, B78	1		DR52, DR15	
6 mo	0	0%	None called	1		DR52, DR4, DR15	
Patients with only class I HLA-Ab detected before transplantation ($n = 2$)							
4							
0	0.5	3.60%	None called	0			Negligible HLA-Ab
5							
0	1		IS	0	—	—	Class I Ab likely were reduced from baseline at 6 mo after transplant.
3 mo	1		IS	0	—	—	
6 mo	0.5	<5%	None called	0	—	—	
12 mo	1		IS	0	—	—	
Patients who developed new HLA-Ab after transplantation ($n = 1$)							
6							
Pre	0	—	—	0	—	—	Although class I Ab became detectable by screening at a gray zone level, the subsequent confirmatory testing suggests that this likely was a false-positive result and that no significant HLA-Ab were present.
3 mo	0.5	<1%	None	0	—	—	
6 mo	0.5	<1%	None	0	—	—	
12 mo	0.5		IS	0		IS	

[a]Ab, antibody; IS, insufficient sample available for rPRA and specificity testing; PRA, plasma renin activity.
[b]For class I and class II screening results: 0 = no antibody detected, 0.5 = gray zone, 1 = positive for antibody.

history of HLA Ab after kidney transplantation, especially in pediatrics. In the adult population, the prevalence of posttransplantation HLA-Ab is documented to be between 11 and 25% (55–60). In a recent study by Terasaki *et al.* (59), 17.8% of patients had HLA-Ab at 1-yr after transplantation. Furthermore, in 2278 prevalent kidney transplant recipients with 1 yr of follow-up, 22% had HLA-Ab (total) and 15% had *de novo* HLA-Ab (59). Notably in this study however, only one patient developed *de novo* HLA Ab after transplantation, and in that patient, the finding was questionable. The absence of *de novo* Ab production may be a favorable prognostic factor for delaying or avoiding CAN. Indeed, the maintenance of excellent GFR in these patients and the lack of interstitial fibrosis in the 1-yr surveillance biopsies may predict excellent long-term graft function.

The number of patients who dropped out of the study was a concern. This outcome would make us reluctant to propose this treatment for routine clinical use or for further study without changes. Of the 13 patients who dropped out of the study, eight did so because of an acute rejection episode. As noted above, the early acute rejection rate was higher than desired, and more potent induction strategies or concomitant chronic immunosuppressives might result in lower rates. Of the eight who dropped out because of acute rejection, three had two episodes

and five had only one, although the protocol permitted the patients to remain on the study regimen after the first acute rejection episode. Importantly, long-term GFR was sustained in the study patients, suggesting that the acute rejection episodes were relatively mild and completely reversed. Of the four patients who dropped out of the study because of adverse events, two had neutropenia. It is possible that dose reduction or the interim use of granulocyte colony–stimulating factor would have permitted those patients to remain in the study. One patient discontinued the study because of diarrhea, vomiting, and fever and substituted tacrolimus for MMF. That patient improved, but similar outcomes might have been obtained if the patient would have substituted azathioprine instead, which was permitted in the study protocol and which had occurred in one other patient. It also is possible that careful concentration control of MMF might alleviate both leucopenia and gastrointestinal complications without adversely affecting outcomes (61). One patient could not tolerate sirolimus in the early posttransplantation period because of poor wound healing; however, that patient was restarted successfully on sirolimus later in his course. The final patient was lost to follow-up. Concerns have been raised recently about the potential for decreased testosterone and increased follicle-stimulating hormone and luteinizing hormone levels in sirolimus-treated male transplant patients (62–64), but the functional significance of those changes are unclear (64) and we do not have data about those hormones in these children.

Of greater concern was the development of PTLD in two patients. Both patients were in the high-risk category of an Ab-negative recipient of a kidney from an Ab-positive donor. One patient had stopped prophylactic ganciclovir 4 mo before the onset of PTLD; importantly, routine surveillance for EBV by PCR had not been instituted as part of the study at that point. Subsequent studies require 12 mo of valganciclovir prophylaxis and frequent monitoring for EBV by PCR, with protocol-determined reduction in immunosuppression when EBV is detected. The second patient with PTLD had other risk factors. That patient had early rejection that was treated with lympholytic Ab and with a change of immunosuppression to tacrolimus, sirolimus, and prednisone, a combination that has been associated with a high incidence of PTLD in children.

We consider the results of this study to represent proof of concept of our proposal that kidney transplantation can be performed in children without the use of CNI. We believe that most of the rejections and complications that were seen in this study could have been avoided by making modifications in the protocol. Most of the acute rejections occurred in the first 6 mo and were cellular (lymphocyte) mediated. Therefore, more substantial induction therapy with a lymphocyte-depleting Ab might be beneficial. Also, some of the early complications of sirolimus might be avoidable by delaying the use of the drug until after the early posttransplantation period. Furthermore, because all three patients with pretransplantation anti-HLA classes I and II Ab had multiple or severe rejection episodes and because the only two graft failures were in this group, it would seem reasonable to include only primary transplant recipients without evidence of Ab sensitization in similar minimization studies in the future.

Conclusion

We conclude that future trials of CNI avoidance or withdrawal in children should be undertaken, with particular attention to sufficient induction treatment that is designed to prevent early acute rejection and perhaps permit a delay in initiation of sirolimus until after the early postoperative period. Other complications of concomitant treatment with sirolimus and MMF should be avoidable through dose adjustment or use of other supportive treatments in the large majority of children. Importantly, all future trials of immunosuppression in children should include safeguards to prevent PTLD, especially in high-risk anti-EBV Ab-negative recipients. The ultimate goal of chronic immunosuppression for children is to use the lowest doses of the fewest possible medications.

Acknowledgments

This study was supported by grants NIH U01 AI46135, PO1 AI50157, and R01 AI54720; Wyeth Pharmaceuticals, Inc.; and NIH NCRR MO1 RR02172 and RR00240.

These results were from a study that was conducted under the auspices of the Cooperative Clinical Trials in Pediatric Transplantation (CCTPT) of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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