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RAPID COMMUNICATION

PROMISING EARLY OUTCOMES WITH A NOVEL, COMPLETE STEROID AVOIDANCE IMMUNOSUPPRESSION PROTOCOL IN PEDIATRIC RENAL TRANSPLANTATION[1]

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Background. **Corticosteroids have been a cornerstone of immunosuppression for four decades despite their adverse side effects. Past attempts at steroid withdrawal in pediatric renal transplantation have had little success. This study tests the hypothesis that a complete steroid-free immunosuppressive protocol avoids steroid dependency for suppression of the immune response with its accompanying risk of acute rejection on steroid withdrawal.**

Methods. **An open labeled prospective study of complete steroid avoidance immunosuppressive protocol was undertaken in 10 unsensitized pediatric recipients (ages 5–21 years; mean 14.4 years) of first renal allografts. Steroids were substituted with extended daclizumab use, in combination with tacrolimus and mycophenolate mofetil. Protocol biopsies were performed in the steroid-free group at 0, 1, 3, 6, and 12 months posttransplantation. Clinical outcomes were compared to a steroid-based group of 37 matched historical controls.**

Results. **Graft and patient survival was 100% in both groups. Clinical acute rejection was absent in the steroid-free group at a mean follow-up time of 9 months (range 3–13.7 months). Protocol biopsies in the steroid-free group (includes 10 patients at 3 months, 7 at 6 months, and 4 at 12 months) revealed only two instances of mild (Banff 1A) subclinical rejection (reversed by only a nominal increase in immunosuppression) and no chronic rejection. At 6 months the steroid-free group had no hypertension requiring treatment ($P=0.003$), no hypercholesterolemia ($P=0.007$), and essentially no body disfigurement ($P=0.0001$). Serum creatinines, Schwartz GFR, and mean delta height Z scores trended better in the steroid-free group. In the steroid-free group, one patient had cytomegalovirus disease at 1 month and three had easily treated herpes simplex stomatitis, but with no significant increase in bacterial infections or rehospitalizations over the steroid-based group. The steroid-free group was more anemic early posttransplantation ($P=0.004$), suggesting an early role of steroids in erythrogenesis; erythropoietin use normalized hematocrits by 6 months.**

Conclusions. **Complete steroid-free immunosuppression is efficacious and safe in this selected group of children with no early clinical acute rejection episodes. This protocol avoids the morbid side effects of steroids without increasing infection, and may play a future critical role in avoiding noncompliance, although optimizing renal function and growth.**

INTRODUCTION

Corticosteroids have been a cornerstone of immunosuppression in organ transplantation for 40 years and are currently the standard in all pediatric renal transplant programs. The burden of steroid use is multisystemic, and the adverse consequences have been greatest amongst pediatric transplant recipients. Despite diligent effort, transplant physicians have, for the most part, struggled unsuccessfully to eliminate the well-recognized steroid complications such as infection, hypertension, hyperlipidemia, growth suppression during crucial growth years, glucose intolerance and diabetes mellitus, bone loss, cataracts, acne, cushingoid appearance, and changes in mood and behavior.

Various attempts have been made to reduce or eliminate steroid use (*1*). Alternate day steroid dosing in selected patients has been shown to achieve some improvement in growth velocity in children (*2*, *3*), but has failed to gain widespread acceptance because of a higher incidence of acute rejection after conversion to alternate day therapy (*4*) and the concerns about reduced compliance with an alternate day drug delivery regime.

In the last 5 years there have been significant advances in immunosuppression, including the introduction of the well-tolerated interleukin-2 receptor inhibitors (daclizumab and basiliximab), mycophenolate mofetil (MMF), sirolimus, and a new formulation of cyclosporine (Neoral). In addition, the calcineurin inhibitor tacrolimus has found increasing use in kidney transplantation. Given these recent advances, complete steroid avoidance in renal trans-

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plantation may be achievable with a well-balanced use of these newer agents.

We report the results of a novel complete steroid avoidance protocol that substitutes corticosteroids with an extended course of daclizumab, in combination with tacrolimus and MMF. To demonstrate the safety and efficacy of this steroid avoidance immunosuppression approach, a single center open labeled phase 1 study was performed in 10 pediatric renal allograft recipients 5–21 years of age. The goal of this trial was to improve the well being of the child and restore normal growth patterns after transplantation, while sustaining good, long-term graft survival and minimizing acute rejection episodes, comparable or lower than our historical incidence of 14% clinical acute rejections at 1 year posttransplantation (*5*).

MATERIALS AND METHODS

Steroid-Free Cohort

Ten unsensitized [panel reactive antibody (PRA)≤10%] recipients (aged 5–21 years) of first renal allografts from either cadaver or living donors, were enrolled into a prospective immunosuppressive protocol of complete steroid avoidance at the Lucile Packard Children's Hospital at Stanford University. Excluded from study entry were sensitized (PRA>10%) recipients, recipients of second or greater renal allografts or patients with exposure to steroids at the time of transplantation. Also excluded from study entry were patients with focal segmental glomerulosclerosis and lupus nephritis as causes of end stage renal disease as most of these patients were on or had recently been exposed to steroids at the time of transplantation. Center institutional review board approval was obtained for the study.

Historical Cohort:

There were 179 transplants performed from 1991–1999 in the Packard program. One hundred ten were first time recipients of kidney transplants on steroid-based immunosuppression and were aged 5–21 years. To allow for more commonality of general management, the most recent historical cohort of 66 first time renal transplant recipients from 1995–1999, aged 5–21 years was examined. After controlling for sex distribution, cause of end stage renal disease, donor source, PRA level, donor and recipient cytomegalovirus (CMV) status, baseline hematocrit, blood pressure, and serum lipid levels, the historical cohort was reduced to 37. The comparison of these 37 patients receiving steroid-based immunosuppression and the 10 steroid-free patients at baseline are shown in Table 1. The living related steroid-based recipients were better matched and 2/37 patients in the steroid-based cohort had PRA levels of more than 10%.

Study Design:

1. End points. The *primary end point* of this study was to assess the effect of complete steroid avoidance on the cumulative incidence of clinical and subclinical acute rejection at *1, 3, and 6 months posttransplantation*. Clinical acute rejection is defined as a more than 10% rise in the serum creatinine with Banff rejection scoring (*6*) on light microscopy of biopsy tissue. Subclinical acute rejection is defined as similar histological evidence of acute rejection on biopsy tissue, without any change in the serum creatinine. Protocol biopsies were done in the steroid free group at 1, 3, and 6 months posttransplantation to assess subclinical rejection in this group (Fig. 1). Twelve month biopsies were performed on four patients who were at this point, and these data are also being presented. Additional nonprotocol biopsies were obtained if clinically indicated (>10% rise in serum creatinine from baseline) at any other time posttransplantation in both cohorts.

Secondary end points were a combination of clinical and laboratory findings, assessed at *6 months posttransplantation* as follows: (1) recipient and graft survival (2); renal function (3) proteinuria (defined as a spot early morning urine protein: creatinine ratio of >0.2) (4); incidence of hypertension (systolic and/or diastolic blood pressures >95th percentile for age) and number of antihypertensive medications (5); incidence of hyperlipidemia (fasting serum cholesterol levels >193 mg/dl and serum triglyceride levels >140 mg/dl) and its treatment (6); cumulative incidence of all posttransplant infections and number of hospitalizations for treatment of infections (7); height delta standard deviation scores (8); body disfigurement (defined as change in body habitus perceived by the patient and the physician on posttransplant clinic visits until the 6th month posttransplant (these were cushingoid facies, acne above baseline pretransplant, hirsuitism, and gingival hyperplasia) (9); incidence of anemia (hematocrit <38% in males and <36% in females) (10), leukopenia (white cell count 4000 μl); and (11) thrombocytopenia(platelet count 150,000 μl); and (12) incidence of other metabolic derangements such as hyperglycemia (fasting blood sugar >115 mg/dl) and metabolic acidosis (serum bicarbonate <20 mEq/liter).

2. Immunosuppression. Immunosuppression consisted primarily of an extended course of daclizumab, tacrolimus, and mycophenolate mofetil (MMF) (Fig. 1). Dacluzimab has never previously been used in renal transplantation for this length of time (up to 6 months posttransplantation), and the rationale for the longer induction period of interleukin- (IL) 2 receptor blockade was to minimize the risk of early acute rejection, in a steroid-free environment. Immunosuppression dosing and target levels are shown in Table 2. Tacrolimus was given orally preoperatively at 0.15 mg/kg/dose for two doses in living donor recipients and one dose in cadaver graft recipients, followed by an i.v. infusion that was initiated intra-operatively (0.07 mg/kg/day) after diuresis was established. Tacrolimus was switched to the oral formulation when oral intake was tolerated. MMF was initiated at 600 mg/m^2/dose i.v., twice a day and converted to an equivalent oral dose when tolerated. Within the first 6 months, patients intolerant of dose reduced MMF received azathioprine. After 6 months, all patients still intolerant of MMF (n=5) were switched to sirolimus with target levels 5–8 ng/ml, as tacrolimus and azathioprine were believed by the investigators to be inadequate long-term combination dual maintenance immunotherapy. A single exception to this immunosuppressive protocol was one patient with partial severe combined immunodeficiency (patient 4). He received dacluzimab and tacrolimus alone and after the sixth posttransplant month has received tacrolimus monotherapy. In steroid-based patients, steroids were initially administered at 1.5 mg/kg/day with a rapid taper to 0.15 mg/kg/day at 4 months posttransplantation.

3. Infection prophylaxis. All steroid-free patients underwent sequential CMV and Epstein-Barr virus (EBV) quantitative polymerase chain reaction (QC-PCR) monitoring and monthly surveillance bacterial urine cultures. All patients received prophylaxis for cytomegalovirus and pneumocystis. Initially only CMV donor antibody-positive/recipient antibody-negative and donor-negative/recipient-positive patients were to receive gancyclovir 5 mg/kg/day i.v. for the first 14 days, followed by 10 mg/kg/dose orally, three times a day for 100 days. After the experience of patient 5 (donor-positive/recipient-positive) who received acyclovir prophylaxis only at 10 mg/kg/dose orally four times a day (see *Infections* in *Results*), all donor or recipient-positive patients, regardless of mismatch, subsequently received gancyclovir. Gancyclovir and acyclovir dosing were adjusted for level of renal function. For pneumocystis prophylaxis, trimethoprim-sulfamethoxazole (TMP/SMX; 2–3 mg/kg of trimethoprim) was given orally, once daily for 4 months. TMP/SMX intolerant recipients received aerosolized pentamidine 300 mg every month for 4 months.

4. Statistical analysis. Patient and graft survival rates were determined by Kaplan-Meyer survival analyses. The difference between survival curves was tested by log-rank tests. Unpaired *t* tests were used for comparison of continuous parametric data between two groups, one-way analysis of variance for more than two groups, and Mann-Whitney *U* test for nonparametric comparison between two

TABLE 1. Patient characteristics and initial immunosuppression at time of transplantation

	Steroid-free	Steroid-based	*P*
No.	10	37	
Females	70%	62.2%	0.28
Mean age	14.4 ± 4.2	16 ± 3.1	0.25
Mean height[a]	145 ± 18.9	156 ± 16	0.14
Height Z score[a]	−1.31 ± 1.25	−1.05 ± 1.39	0.61
Mean weight	49.3 ± 28.8	49.8 ± 15.9	0.95
Mean BSA	1.42 ± 0.5	1.46 ± 0.3	0.75
Race: Caucasian	60%	56.7%	0.74
Cause of end stage renal disease:			
Chronic glomerulonephritis	30%	30%	1.0
Renal dysplasia and reflux	30%	30%	1.0
Obstructive uropathy	20%	21%	0.81
Other (polycystic disease, Wilms', etc.)	20%	19%	0.87
LRD	80%	81%	0.94
2-Haplotype match	0%	3%	<0.0001
1-Haplotype match	75%	97%	0.02
0-Haplotype match	25%	0%	<0.0001
CAD	20%	19%	0.94
Mean HLA antigen match	1.5 ± 0.5	0.85 ± 0.6	0.51
PRA > 10%	0%	5%	<0.05
CMV:			
Donor+/recipient−	20%	24.3%	0.51
Donor+/recipient+	50%	43.3%	0.41
Donor−/recipient+	10%	10.8%	0.87
Donor−/recipient−	20%	21.6%	0.81
EBV: recipient− (donor status not always known)	20%	24.3%	0.61
Mean systolic BP	135.6 ± 20	141 ± 14	0.41
Mean diastolic	77.7 ± 11.9	81.9 ± 11.9	0.37
Mean hematocrit[a]	34.5 ± 9.3	34.1 ± 6.3	0.88
Mean serum cholesterol	169.3 ± 35.6	178.3 ± 46	0.51
Mean serum triglyceride	193.6 ± 97.6	199.5 ± 101.8	0.87
Initial immunosuppression			
Induction therapy			
Dacluzimab	100%	37.8%	<0.0001
ALG/ATG	0%	8%	<0.0001
Maintenance (at 6 mo post-txp)			
Steroids	0%	100%	<0.0001
Tacrolimus	100%	13.5%	<0.0001
Cyclosporine A	0%	86.5%	<0.0001
MMF	90%	51%	<0.0001

[a] These values were assessed for all steroid free patients, apart from patient 4 for reasons described in the text.

groups. Paired *t* tests were used for testing repeated measures with parametric data and the Wilcoxon signed-rank test for nonparametric data. Pearson's correlation coefficient was used for analysis of correlations with parametric data and the Spearman rank correlation coefficient for nonparametric correlations. $P<0.05$ was considered significant. Means are reported as mean±SD.

RESULTS

A. Length of Steroid-Free Cohort Follow-Up at Time of Analysis:

The mean follow-up period is 9±3.8 months for the cohort (range 3–13.7 months). Four patients are at 1 year or more, 5 are at 9 months, 7 at 6 months, and all 10 are more than 3 months posttransplantation at the time of this report (Fig. 2).

B. Data Analysis: Primary study end points

1. Incidence of clinical acute rejection. There were no clinical acute rejections at 1, 3, and 6 months post transplanta-

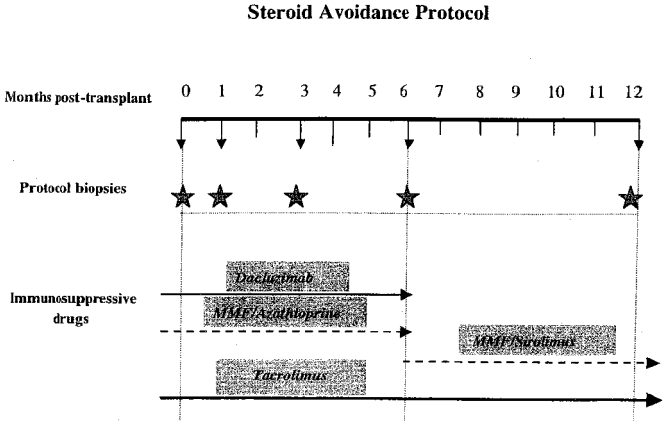

FIGURE 1. The steroid avoidance protocol is shown over the first posttransplant year, with times of protocol biopsies (0, 1, 3, 6, and 12 months) and immunosuppressive drug usage.

TABLE 2. Immunosuppressive drug doses/trough levels in the steroid-free cohort

Drugs	Pre-op	Post-op
Dacluzimab	2 mg/kg	1 mg/kg: wk 2, 4, 6, 8, 11, 15, 19, 23
Tacrolimus	0.15 mg/kg/dose, bid	To target trough levels (ng/ml)
	Wk 0–2	17–20
	Wk 3–4	15–17
	Wk 5–8	12–15
	Wk 9–12	10–12
	Wk 13–16	8–10
	Wk 17–20	5–7
MMF	600 mg/m2/dose, bid	600 mg/m2/dose × 2 wk, 450 mg/m2/dose × 4 wk, 300 mg/m2/dose thereafter

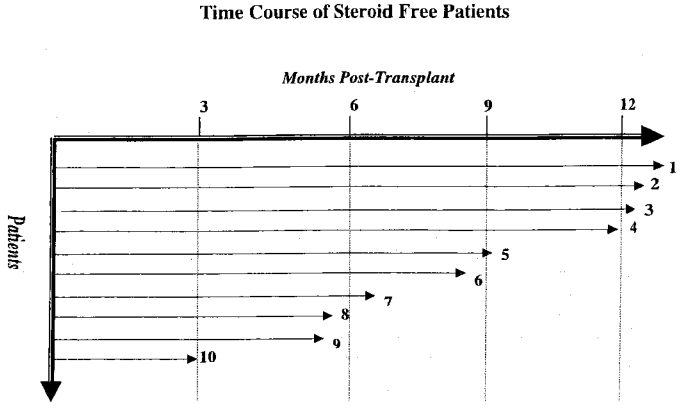

FIGURE 2.

tion and serum creatinine values were stable in all study patients.

2. Incidence of Subclinical Acute Rejection (as Assessed by a Total of 41 Protocol Biopsies)

Protocol biopsies were only done in the steroid free group. All protocol biopsies were examined by light microscopy for evidence of interstitial mononuclear infiltrate and tubulitis (to suggest acute rejection) and tubular atrophy, fibrosis, and arteriolar hyalanosis (to suggest drug toxicity and chronic damage). There was one case of drug toxicity and two subclinical acute rejections overall. None of the patients was treated with our standard three-dose steroid pulse therapy. There were no biopsy proven chronic rejections. As protocol biopsies were not performed in the steroid-based group, subclinical acute rejection could not be longitudinally assessed in these patients.

3. Results of Protocol Biopsies

(i) One month protocol biopsy data. One patient had mild tacrolimus toxicity (patient 2 had high tacrolimus 12 hr trough levels for the first month at 21.7±5.8 ng/ml). Changes of tubular vacuolization on the 1 month biopsy were reversed on the 3 and 6 month biopsies after lowering the tacrolimus levels. One patient had Banff 1A mild tubulitis (patient 3). This subclinical rejection resolved by increasing the dose of MMF (300–450 mg/m^2/dose) and the subsequent 3- and 6-month biopsies revealed no evidence of acute rejection.

(ii) 3 month protocol biopsy data. No acute rejections or drug toxicity.

(iii) 6 month protocol biopsy data. One patient had Banff 1A mild tubulitis (patient 5). This subclinical rejection was treated by a single 10 mg/kg solumedrol pulse, followed by a change in maintenance immunosuppression from low dose MMF (300 mg/m^2/dose) to sirolimus (target trough levels 5–8 ng/ml), as this patient was unable to tolerate an increase in MMF dosing due to leukopenia. This patient weighs 83 kg and the serum creatinine continues to be very stable at 0.8 mg/dl at 9 months posttransplantation.

(iv) 12-month protocol biopsy data. These data are thus far available from only four patients (patients 1, 2, 3, and 4). There are no subclinical acute rejections, but three of the four biopsies (patients 1, 2, and 3) have mild chronic tacrolimus toxicity, evidenced by minimal striped interstitial fibrosis and tubular atrophy. Patient 4 is on tacrolimus monotherapy because of partial severe combined immunodeficiency, and the other three are receiving tacrolimus and sirolimus.

Secondary end-points. Secondary end points have been obtained at 6 months posttransplantation. Patient 4 in the steroid free group has primordial dwarfism, transfusion-dependant bone marrow hypoplasia, and partial severe combined immunodeficiency, and therefore has been excluded from analysis of growth outcomes and anemia in this study.

1. Patient and graft survival. Patient and graft survival was 100% in both groups at 6 months posttransplant.

2. Renal function. Apart from a single patient with delayed postoperative ATN after immediate excellent function (patient 9 had ATN at 48 hr due to hypotension), none of the steroid-free patients had clinical graft dysfunction (as defined by >10% rise in the serum creatinine above baseline). All patients have maintained steady serum creatinine levels. Comparative analysis of graft function in the steroid-based group, shows that five patients (13.5%) had clinical graft dysfunction by 6 months posttransplant. Of these, one patient (2.7%) had acute rejection on biopsy and the remaining four (10.8%) had calcineurin inhibitor toxicity on biopsy. Of the latter four patients, one was on tacrolimus and the remainder on cyclosporine A; one of these patients with cyclosporine A-induced drug toxicity also had chronic rejection on biopsy (2.7%). Serum creatinine levels and Schwartz creatinine clearance (in ml/min/1.73 m^2) were better in the steroid-free group at 3 months posttransplantation (serum creatinine 0.93±0.39 vs. 1.30±0.59; P=0.05 and Schwartz clearance 92±29.2 vs. 77.6±14.3; P=0.04, between the steroid-free and steroid-based groups, respectively). This was seen despite maintaining the steroid-free patients on higher tacrolimus trough levels until the fourth month posttrans-

plant, by which time the levels were statistically equivalent between the two groups under study (Table 3). Renal function was comparable between the 2 groups at 6 months posttransplantation. Patient 9 in the steroid-free group had biopsy proven delayed postoperative ATN (not requiring dialysis) and focal renal cortical infarction from severe postoperative hypotension, and has not been excluded from comparative analysis with patients in the steroid-based group, none of whom had delayed graft function or ATN. This patient weighs 101 kg and has a serum creatinine of 1.5 mg/dl at 5 months posttransplant, the highest creatinine in the steroid-free series.

3. Proteinuria. A total of 16.2% of patients in the steroid-based group, and no patients in the steroid-free group had proteinuria at 6 months posttransplantation. This difference is not statistically significant ($P=0.56$), but the complete absence of proteinuria in the steroid-free group is noteworthy.

4. Hypertension. There was a significant increase in the incidence and the severity of hypertension and the number of medications required for blood pressure control in the steroid-based group, regardless of the calcineurin inhibitor agent used (Table 4). In the steroid-based group, at 1 month 63.3% were on two or more antihypertensive agents ($P=0.004$); at 3 months 66.6% were on two or more antihypertensive agents ($P=0.002$); and at six months this decreased to 45.9% ($P=0.01$). All steroid-free recipients on antihypertensive drugs received single drug therapy, the most commonly used drug being a calcium channel blocker (nifedipine or isradipine). Hypertension had completely resolved in all steroid-free patients by the sixth posttransplant month, whereas 70.2% of steroid-based patients remained hypertensive, including three of five receiving tacrolimus ($P<0.001$). The mean number of antihypertensive drugs needed to achieve normotension were significantly different between the two cohorts at 1, 3, and 6 months posttransplant ($P<0.001$).

5. Hyperlipidemia. Mean serum cholesterol levels were significantly higher in the steroid-based group (Table 5), as none of the children on steroid-free immunosuppression had high serum lipid levels. A total of 54% (20/37) of steroid-based children continued to have serum cholesterol levels above the normal range at 6 months posttransplant ($P=0.01$), when steroid dosing was at baseline levels of 0.15 mg/kg/day. All 20 of these patients were on cyclosporine A ($P<0.0001$). Five (13.5%) of these patients were on lipid lowering drugs at 6 months posttransplantation ($P<0.0001$). The mean serum triglyceride levels were higher early posttransplant in the steroid-based group, but these levels did not approach significance (Table 5). At 6 months, 64.8% (24/37) of steroid-based children had sustained hypertriglyceridemia. Of these 24 patients, 21 were on cyclosporine A and 3 were on tacrolimus, showing no treatment bias for hypertriglyceridemia ($P=0.47$).

TABLE 4. Prevalence of hypertension in steroid free patients and their controls

Mo posttransplant	Steroid free	Steroid based	*P*
1	40% (4/10)	81.1% (30/37)	0.024
3	10% (1/10)	73.0% (27/37)	0.0009
6	0% (0/7)	70.2% (26/37)	0.003

6. Infections. Patient 5, who had multiple bacterial and fungal infections (urosepsis and septicemia from infected dialysis access) pretransplantation, had systemic (lung and kidney) CMV infection at 5 weeks posttransplantation. She received acyclovir prophylaxis for CMV donor-positive/recipient-positive immune status. The remainder of the steroid-free patients received gancyclovir prophylaxis. Patient 4 has partial severe combined immunodeficiency and on surveillance QC-PCR was noted to have a high CMV viral load at 6 weeks posttransplantation, and never manifested systemic CMV. Reduction in viral load was achieved by gancyclovir treatment i.v. for 3 weeks and orally for 3 months. Two steroid-based patients had systemic CMV at 1 month posttransplantation, but based on the clinical practice at that time, no steroid-based patients had surveillance CMV QC-PCR, hence information on CMV viral loads for this group was not available. None of the steroid-free patients had systemic EBV infection, posttransplant lymphoproliferative disorder (PTLD), or increased viral loads by EBV QC-PCR. Again, viral load for EBV could not be assessed in the steroid-based group as this test has only recently been incorporated into routine clinical care for our patients. Herpes simplex (HSV-1) stomatitis was seen in three steroid-free patients (patients 2, 3, and 7), who were all on sirolimus therapy and responded to topical acyclovir cream (n=2) and oral acyclovir (n=1). HSV-1 oral infection was not noted in the steroid-based group under study. TMP/SMX was used initially for pneumocystis prophylaxis, but was switched to inhaled monthly pentamidine primarily because of leukopenia (see *Leukopenia* below). Among the steroid-free patients, 6/10 (60%) were on pentamidine, verses 2/37 (5%) in the steroid based group ($P<0.001$).

The incidence of mild bacterial infections (not requiring hospitalization) was not different and there were no wound infections or bacterial pneumonias in the steroid-free group. The number of patients requiring hospitalizations at 6 months for treatment of infection was also not significantly different in the two groups studied (three in the steroid-free group verses seven in the steroid-based group; $P=0.15$). Causes of hospitalization in the steroid-free cohort were rotavirus gastroenteritis and stomatitis in patient 2 (17 days), central line sepsis and parotitis in patient 4 with immunodeficiency (11 days), and systemic CMV in patient 5 (27

TABLE 3. Comparison of renal function in steroid-free and control patients

Mo posttransplant	Mean serum creatinine (mg/dl)			Schwartz creatinine clearance (ml/min/1.73 m2)		
	Steroid free	Steroid based	*P*	Steroid free	Steroid based	*P*
1	0.91 ± 0.31	1.13 ± 0.33	0.06	90.4 ± 27.9	87.2 ± 19.4	NS
3	0.93 ± 0.39	1.30 ± 0.59	0.05	92.0 ± 29.2	77.6 ± 14.3	0.04
6	0.95 ± 0.41	1.26 ± 0.45	NS	93.8 ± 41.2	77.8 ± 18.9	NS

TABLE 5. Cholesterol and triglyceride values in steroid-free and control patients

Mo posttransplant	Mean serum cholesterol (mg/dl)			Mean serum triglycerides (mg/dl)		
	Steroid free	Steroid based	*P*	Steroid free	Steroid based	*P*
1	158.4 ± 33.2	232.3 ± 49.4	0.0001	141.0 ± 65.2	151.6 ± 68.7	NS
3	157.6 ± 31.1	211.9 ± 42.9	0.001	165.8 ± 82.1	197.5 ± 107.5	NS
6	146.8 ± 22.7	202.3 ± 42.5	0.007	196.0 ± 108.0	189.8 ± 87.4	NS

days). Total hospital days for infection in the steroid-based group were 83 days.

7. Height Z scores and delta height Z scores. Mean delta height Z scores trended better in the steroid-free group at 6 months post transplantation (0.08±0.1 in the steroid-free vs. 0.003±0.1 in the steroid-based group; $P=0.07$), but the follow-up period is currently too short to show significant differences.

8. Change in body mass index (BMI delta). As early as 1 month posttransplant, there was an increase in the body mass index (BMI) in children receiving steroids, versus a decrease in steroid-free patients (mean BMI delta: −0.21±0.83 in steroid-free vs. 1.2±1.26 in steroid-based; $P=0.05$). A difference was still apparent at 3 months (mean BMI delta: 0.2±0.7 in steroid-free vs. 1±1.2 in steroid-based; $P=0.05$). By 6 months, as the steroid dose was lowered, the mean BMI delta became more comparable in the two groups (0.24±0.7 vs. 0.53±1.1; $P=0.08$).

9. Body disfigurement. There was a significant difference in incidence of cushingoid facies, acne, hirsuitism, and gingival hyperplasia between the steroid-free and steroid-based patients (Table 6). The latter two side effects appear to be due to cyclosporine A, which was not used in the steroid-free group of patients. The cushingoid facies and acne were notable despite the use of a low-dose steroid-based protocol.

10. Anemia. Despite comparable hematocrit levels and iron reserves pretransplant between the two groups, the steroid-free patients had delayed recovery of anemia in the posttransplant period. Steroid-free patients required significantly higher use of erythopoeitin therapy (Table 7). The use of erythopoeitin brought the hematocrit in the steroid-free patients in line with the steroid-based patients by 6 months (33.6±4.6 vs. 34.4±2.9%, respectively). At 6 months, all steroid-free patients were still on erythopoeitin, whereas none of the steroid-based patients needed erythopoeitin at this time ($P=0.0001$). The mean dose of erythopoeitin at 6 months in the steroid-free group was 257.8 U/kg/week (range 76–515 U/kg/week). Despite oral iron supplementation, the steroid-free group trended toward lower mean serum ferritin levels (38 ng/ml), compared with steroid-based patients (88 ng/ml) at 6 months, though this difference was not significant ($P=0.07$).

11. Leukopenia. The white blood cell counts also trended lower in the steroid-free group at 6 months (Table 8) and three of nine of patients in the steroid-free group (excluding analysis of patient 4) needed intermittent granulocyte colony-stimulating factor (G-CSF) to maintain white cell counts >4.0. Leukopenia in the steroid-free group correlated with use of MMF ($P<0.05$). In the steroid-based group, there was no correlation between low white blood cell counts and MMF use and none of these patients needed G-CSF.

12. Thrombocytopenia. There was no difference in mean platelet counts in the two groups overall. Four patients (patients 1, 2, 3, and 6) in the steroid-free group on sirolimus trended toward lower platelet counts (mean counts 161,000 μl in these four patients vs. 294,000 μl in the remainder; $P=0.09$).

13. Hyperglycemia and metabolic acidosis. There were no differences between the incidence of hyperglycemia in both groups. The mean blood glucose levels were 96±17 and 99.4±16.5 mg/dl in the steroid-free and steroid-based group at 3 months and 93.4±13.7 vs. 107.2±25.9 mg/dl, respectively, between the two groups at 6 months. The incidence of metabolic acidosis was similar in the two groups at 30% each, with 80% of the steroid-based acidotic patients on cyclosporine A. Bicarbonate replacement requirement was also similar at 0.6±0.7 vs. 0.4±1 mg/kg/day, in the steroid-free and steroid-based groups.

TABLE 6. Comparison of body disfigurement posttransplant between the steroid free and steroid based groups

Cosmetic side effects of immunosuppression	Steroid free	Steroid based	*P*
Cushingoid facies	0%	100%	0.0001
Acne above baseline pre-txp	10%	76%	0.0001
Hirsuitism	0%	80%	0.0001
Gingival hyperplasia	0%	83%	0.0001

DISCUSSION

Initial results from this prospective study suggest that kidney transplantation in this selected group of children without any steroids is safe with excellent early graft function and graft survival, no clinical acute rejections, a low incidence of borderline subclinical acute rejections, and a significant reduction of hypertension, hyperlipidemia, and body disfigurement in the early posttransplant period.

Currently only 26% of children with functioning grafts for more than 4 years posttransplant are on an alternate-day steroid dosing regimen (*7*). A few efforts have focused on steroid withdrawal after some defined period, with concomitant calcineurin inhibitor use, to reduce hypertension, hypercholesterolemia, cushingoid habitus, and to boost growth of children posttransplantation. Steroid withdrawal has been associated with a significant risk of acute rejection in the short term, and allograft loss in the long term (*4*). The aggressiveness of the steroid taper to the point of withdrawal is dependant on the transplanted organ, and has been most successful in liver recipients, where steroids have been withdrawn as early as 3 months posttransplant without increased risk of acute rejection (*8*). The risks of acute rejection with steroid withdrawal are far greater in renal transplantation because of the greater immunogenicity of the kidney, and may be as high as 75% (*1*). Consequently, although many transplant programs now use lower steroid doses, few adult programs and even fewer pediatric kidney transplant programs currently practice steroid withdrawal.

TABLE 7. Comparison of anemia in patients receiving steroid free immunosuppression with the control group

	1 mo		3 mo		6 mo	
	HCT (vol%)	EPO therapy (%)	HCT (vol%)	EPO therapy (%)	HCT (vol%)	EPO therapy (%)
Steroid free	26.8 ± 3.7	62.5	29.8 ± 3.4	57.1	33.6 ± 4.6	100
Steroid-based	32.1 ± 3.2	2.7	33.4 ± 4.1	2.7	34.4 ± 2.9	0
p-value	0.004	0.0001	NS	0.0001	NS	0.0001

Anemia is defined as a hematocrit >2 SD below the mean for age. HCT, Hematocrit; EPO, erythropoietin.

TABLE 8. Comparison of mean white cell counts and platelet counts post transplant in the steroid based and steroid free groups

Post transplant months	Mean white blood cell count (cells/mm^3)		
	Steroid free	Steroid based	*P*
1	9.0 ± 1.4	11.5 ± 5.0	0.01
3	4.8 ± 1.3	8.7 ± 3.4	0.0003
6	4.4 ± 1.1	7.9 ± 3.0	0.01

Complete steroid avoidance in kidney transplantation has been attempted more recently with some success in adults (*9*). The rates of early acute rejection are comparable in these latter trials with the respective individual center's acute rejection rates on steroid-based immunosuppression (20–30%). There is essentially no experience, however, with complete steroid avoidance immunosuppressive protocols in children, the patient cohort that would benefit the most from a steroid-free approach. As most adult and pediatric renal transplant programs have lowered cumulative acute rejection rates to 15–20% at 6 months posttransplant with current immunosuppressive protocols, new attempts at steroid minimization protocols will not be therapeutically acceptable if they are encumbered with higher acute rejection rates and graft loss.

We propose that steroid dependency cannot develop in a patient not previous exposed to steroids. If suppression of the immune response becomes conditioned to steroid exposure, then subsequent steroid withdrawal has the potential for a detrimental outcome, as has already been demonstrated by a number of clinical experiences (*4*, *9*). In addition, steroids appear to induce a degree of peripheral tolerance by a Fas-mediated mechanism (*10*). Steroid withdrawal after previous steroid exposure can therefore theoretically induce withdrawal of this possible tolerogenic response, increasing the risk of acute graft rejection. In other words, a steroid-adapted suppression of the immune response is destined to increase the risk of acute rejection on attempted steroid withdrawal. This is most pertinent in an immunogenic organ, such as the kidney. We believe that a completely steroid-free immunosuppressive milieu from the beginning should, by exclusion, not give rise to a steroid-dependant suppression of the immune response, which makes either steroid withdrawal or alternate dosing hazardous for rebound acute rejection. Late steroid withdrawal (after the first transplant year) has been successful in a small number of patients with stable graft function (*11*), but the small target population that can benefit from this approach may be difficult to predict and remains limited.

There were no clinical acute rejection episodes in our steroid-free patients. Frequent protocol biopsies were conducted in this study and have shown only a low incidence of very mild subclinical acute rejection by 6 months posttransplantation and absence of this finding in the four patients who were biopsied at 1 year posttransplantation. Subclinical acute rejection with notable tubulitis has been shown to be an imposing problem in steroid-based patients with an incidence as high as 30% in the first 3 months post transplant (*12*), yet our steroid avoidance protocol had only a 10% incidence for this observation at 3 months.

Our steroid-free cohort demonstrated a statistically beneficial effect on early graft function with better renal clearance at 3 months posttransplant. A larger percentage of the steroid-free group were on aerosolized pentamidine for pnemocystis prophylaxis, and it cannot be ruled out that treatment with TMP/SMX may have raised serum creatinine concentrations and lowered creatinine clearance in the first 4 months posttransplantation. Greater patient entry and longer follow-up is needed to assess a sustained beneficial effect on longer-term graft function in the steroid-free group. Nevertheless the complete absence of proteinuria and chronic rejection at 6 months, and only two Banff 1A subclinical acute rejections, suggests that the combination and balance of immunosuppressive drugs in our steroid-free immunosuppressive regimen may be efficacious at preventing subclinical acute rejections and chronic rejection, with subsequent better preservation of graft function. Again, additional patient entry and longer follow-up is needed to validate this observation.

We have also taken note of the mild tacrolimus toxicity noted in some of our biopsies and are now reducing our initial tacrolimus target levels. It is unclear whether the absence of steroids may have contributed to the minimal interstitial fibrosis observed on the 1-year biopsies. However, chronic allograft nephropathy can occur in two-thirds of steroid-based cyclosporine or tacrolimus treated kidney allograft recipients at 2 years posttransplantation, with 24% of tacrolimus-treated patients exhibiting definite nephrotoxicity with hyaline arteriolar change, sometimes accompanied by isometric vacuolization of the tubules (*13*). None of our 1-year protocol biopsies exhibited the latter two changes. In an Australian report, 3-month protocol biopsies demonstrated chronic allograft nephropathy in 24% of patients (*14*). We had no evidence of any such changes in our 3-month protocol biopsies.

Viral infections may be slightly higher in the steroid-free group. One patient in the steroid-free group had a pretransplant history of multiple severe infections and developed early clinical systemic CMV while receiving acyclovir prophylaxis. As the CMV occurred at 5 weeks posttransplant, it was within the period covered by the standard use of dacluzimab, whereas a longer course of this drug has thus far not proven

causative of clinical CMV infection in our series. With the above patient experience, we have since then changed our prophylaxis for CMV by administering gancyclovir in all recipient or donor CMV seropositive states. Patient 4, with partial severe combined immunodeficiency with high peripheral blood CMV copy number by QC-PCR, was treated with gancyclovir and did not manifest systemic disease. The incidence of bacterial infections or rehospitalizations was no different between the steroid-free and steroid-based groups.

Significant beneficial effects were seen with regards to blood pressure and lipid profiles in the steroid-free group. Most importantly none of the steroid-free patients required any antihypertensives at 6 months posttransplant. Before 6 months, steroid-free recipients were only on single antihypertensive therapy (40% at 1 month and 10% at 3 months), whereas more than two-thirds of the steroid-based patients were on two or more antihypertensives. At 6 months approximately one-half of the steroid-based patients still needed dual or triple antihypertensive drugs for effective blood pressure control. The calcineurin inhibitor of choice in the steroid-based group was cyclosporine A (32/37), rather than tacrolimus (5/37), the latter being the only calcineurin inhibitor in the steroid-free group. As our control steroid-based cohort had significant hypertension, regardless of the choice of calcineurin inhibitor therapy, the observed significantly beneficial effect on blood pressure in the steroid-free group seems to be due to the complete avoidance of corticosteroids. This is further underscored by the fact that we maintained higher trough levels of tacrolimus over this period, when compared with children on steroid-based immunosuppression.

Hyperlipidemia was a significant problem in the steroid-based group, with more than one-half of the patients having sustained hypercholesterolemia and about two-thirds of the children having sustained hypertriglyceridemia at 6 months posttransplant. All children with hypercholesterolemia were on cyclosporine A, which is known to more likely cause hyperlipidemia than tacrolimus (*15*). The absence of hyperlipidemia in the steroid-free group may be related to the complete absence of cyclosporine A, but the patient numbers are currently too small to ascertain the additive effect of a steroid-free enviorenment. Recent studies have shown that even late steroid withdrawal has a beneficial effect on arterial hypertension and hyperlipidemia (*16*, *17*), but with an increased risk of acute rejection episodes. None of the steroid-free group had hyperlipidemia at any point post transplant, despite five patients being on sirolimus therapy. Likely explanations for this are: (a) sirolimus trough target levels were relatively low (5–8 ng/ml) when sirolimus was introduced as maintenance therapy at six months posttransplantation in all five patients, although higher drug levels are more likely to result in hyperlipidemia (*18*) and (b) all steroid-free patients were on tacrolimus, which has a lower risk of hyperlipidemia, when compared to a sirolimus-cyclosporine A combination (*19*, *20*).

It is well recognized that clinical acute rejections and subclinical rejections are likely immune risk factors for chronic rejection in renal allografts, whereas hypertension and hyperlipidemia are two of the leading nonimmune risk factors for chronic rejection. A significant reduction of these risk factors in the steroid-free patients suggests a likely reduction in the chronic rejection injury and projects probable prolonged allograft life. The absence of proteinuria in the steroid-free patients at 6 months also appears to support this observation. Longer follow-up is needed to confirm this potential allograft advantage.

Six-month analysis of growth reveals positive growth trends in the steroid-free children over those with steroid-based immunosuppression. Longer follow-up is also needed to gain better insights into improved growth patterns in children with growth potential, in a steroid-free versus a steroid-based environment.

Anemia and lower white blood cell counts were seen in the steroid-free group, with a greater dependency on erythropoietin and G-CSF for value normalizations. This may be due to a lack of a steroid bone marrow boosting effect on erythro- and myelopoeisis, as steroid treatment has been shown to increase reticulocytes, red cell counts, and hemoglobin levels, as well as increase the gastric absorption of vitamin B12 (*21*). Steroids may also temper the myelosuppressive effect of MMF. The anemia and leukopenia finding has not been seen in the adult steroid-free trials.

The difficulty in maintaining all steroid-free patients on MMF is probably related to two factors: 1) the gastrointestinal side-effects of MMF may be additive with concomitant use of tacrolimus, which can also cause similar disturbances; and 2) the absence of steroids appears to allow the potential myelosuppressive effects of MMF to become more manifest.

Intermittent noncompliance with medications is one of the major causes of chronic rejection in any pediatric transplant program and leads to gradual loss of graft function after 3–5 years posttransplantation (*22*). The importance of the lack of cosmetic side effects in the steroid-free patients cannot be minimized. The increase in weight and body mass index was minimal in the steroid-free group. None of the steroid-free children had a change in their facial appearance with mooning, or change in their body habitus with the development of the well-known buffalo hump seen with high-dose steroids. None of them were plagued with the severe acne that often accompanies high dose steroids and can be psychologically crippling for a young teenager. The use of tacrolimus in the protocol kept all children free of hirsuitism and gingival hyperplasia. Compliance with this immunosuppressive regime is thus likely to be greater than with any steroid-based protocol.

In conclusion, the debate about early versus late steroid withdrawal in steroid-based immunosuppressive protocols may become redundant if the early promising results with our complete steroid avoidance protocol are further validated with longer patient follow-up and greater patient entry. Our protocol appears to have the potential to achieve the highly desired universal objectives of pediatric renal transplantation: quality graft survival and function, low incidence of acute rejection and infection, and avoidance of posttransplantation hypertension, hyperlipidemia, and body disfigurement. The early clinical acute rejections noted in the adult steroid avoidance studies have thus far been avoided. More definitive conclusions regarding our novel complete steroid avoidance immunosuppression protocol, such as improved growth potential, cannot be drawn at this time and also await greater patient entry and longer follow-up data. Until the latter are achieved, these excellent preliminary results should be viewed with caution. We are now proceeding with this protocol in infants and small children less than 5 years of age, where this protocol may have its most salutary effect.

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