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A Randomized Pilot Study of Donor Stem Cell Infusion in Living-Related Kidney Transplant Recipients Receiving Alemtuzumab

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Abstract

Background—Transplant tolerance would remove the need for maintenance immunosuppression while improving survival and quality of life.

Methods—A prospective, randomized pilot study was undertaken to assess the safety and efficacy of donor stem cell infusion (DSCI) in living-related kidney transplant recipients treated with alemtuzumab (C1H) induction and tacrolimus and mycophenolate maintenance with switch to sirolimus and weaning over 2 years.

Results—Four patients received DSCI; 5 patients were controls. Graft failure occurred in 2 patients in the DSCI arm. Recurrence of glomerular disease occurred in 2 DSCI recipients, leading to graft loss in one. Biopsy-proven acute rejection episodes occurred in 3 patients (2 in the DSCI vs. 1 in the control). One DSCI patient, with recurrence, subsequently developed antibody-mediated rejection leading to graft failure. In the remaining 2 DSCI patients, weaning was attempted but was not successful. All (4/4) DSCI patients had biopsy-proven chronic allograft injury and/or recurrence.

Conclusion—DSCI with C1H induction and a steroid-free maintenance regimen in a small group of patients failed to induce tolerance, with suboptimal patient and graft survival. The results do not justify extension of this particular trial and underscore the importance of patient selection, specifically avoidance of patients with glomerulopathies whose recurrence may obscure potential benefit.

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Competing Interests: The authors declare no other funding or conflicts of interest.

Keywords

donor-derived hematopoietic stem cells; alemtuzumab; allograft tolerance; living-related kidney transplantation

Introduction

Despite recent advances in kidney transplantation, life-long immunosuppression can cause significant long-term side effects. Transplant tolerance would establish donor-specific unresponsiveness without maintenance immunosuppressive medication while preserving intact immune function against infections, and potentially improving graft and patient survival. One such strategy has been to establish persistent donor-specific chimerism in the host, facilitated by initial depletion of immune cells and introduction of donor cells to the host during the peritransplant period.[1-7] Alemtuzumab (C1H) is known for its potent and long-lasting lymphocyte depleting effects[8] without interfering with major T-cell signaling pathways that may play a role in tolerance induction.[9] It has also been suggested that C1H facilitates the near tolerance state in kidney transplant recipients when used with low-dose cyclosporine.[10] We hypothesized that C1H, in combination with perioperative stem cell infusion, would increase the probability of tolerance induction.

Results

A total of 9 patients were enrolled into this prospective randomized pilot study. Four patients who were randomized for the study protocol received 2 doses of C1H induction and donor stem cell infusion (DSCI), while 5 control patients received C1H induction without DSCI. Both groups were initially maintained with tacrolimus and mycophenolate mofetil (MMF) without maintenance corticosteroids (Figure 1a and 1b). Table1 summarizes the demographic characteristics, primary diagnoses and overall patient and graft outcomes of all cases. The DSCI group consisted of 4 patients with a median age of 27yrs (range:19-64) and the non-DSCI control group consisted of 5 patients with a median age of 34yrs (range: 18-40). Median BMI was 21kg/m^2 (range:18-32) in the DSCI group and 25kg/m^2 (range: 17-28) in the control group. All donors were first-degree relatives, with median ages of 42yrs (range:25-55) and 41yrs (range:32-53) in the DSCI and control groups, respectively. No significant differences were noted in terms of demographics between the two groups. All patients received C1H as scheduled without major complications during or after C1H infusions. Profound lymphocyte depletion was noted in the peripheral blood after C1H administration. Figure 2 shows the post-transplant evolution of T-lymphocytes (CD3+), B-lymphocytes (CD19+), NK-lymphocytes (CD56+), and regulatory T-lymphocytes (CD4+CD25high) in the recipients' peripheral blood as measured by flow cytometry. No statistically significant differences were observed in depletion between the DSCI and control groups.

On day 5 and 4 months after the kidney transplantation, the patients in the DSCI group received a median of 3.8×10^7 CD34+ cell infusion isolated from the same donor as the kidney graft. The patients tolerated the DSCI infusion procedure well without side effects. No clinical or laboratory evidence of graft-versus-host disease (GVHD) was seen in any of recipients who received DSCI. All donors tolerated the nephrectomy and leukapheresis well except for some bone pain attributed to pegfilgrastim administration. One donor was not subject to leukapheresis at month 4 because a decision was made to not perform a second DSCI with the recipient due to severe primary disease recurrence.

Figure 3 compares individual serum creatinine levels over time in the DSCI and control groups from the transplant to the last follow-up or graft failure (if it occurred); major clinical events were also marked on the time scale. Mean creatinine levels of the patients with functioning grafts were 1.25mg/dl in the DSCI group and 1.4mg/dl in the control group at the last follow-up. Attempts to wean maintenance immunosuppression were made with 2 DSCI patients who did not develop disease recurrence; no weaning attempt was made in other two patients because of recurrent original disease (Table 1). In one patient (DSCI #3), tacrolimus+MMF was converted to sirolimus+MMF at month 10, and sirolimus was stopped at month 26. This patient has remained on MMF 1gram twice daily through his last follow-up at post-transplant month 50 because of persistent proteinuria (>3gram/24hr). The other patient (DSCI #4) underwent weaning from tacrolimus+MMF to sirolimus+MMF at month 4 and sirolimus was discontinued at month 21, remaining only on MMF. However, the patient developed a Banff IA rejection at month 24 and returned to a full maintenance immunosuppression regimen with the addition of corticosteroids until the last follow-up at post-transplant month 43.

Two patients (50%) in the DSCI group experienced biopsy-proven acute rejection (BPAR) episodes. DSCI #1 (Table 1 and Fig 3) experienced her first BPAR episode at post-transplant month 47; this Banff IA rejection was treated by steroid pulse. This patient developed another BPAR episode with a humoral component (C4d+ and *de novo* DSA) at month 52 and was treated with Thymoglobulin (7 mg/kg), intravenous immunoglobulin, and one dose of rituximab (375mg/m^2). She developed her third BPAR at 55 months, which was treated with intravenous immunoglobulin and steroid pulse. After the last episode, the patient developed viral encephalopathy with BK viremia (negative SV40 staining on graft biopsy) and returned to dialysis 56 months post-transplant. The other patient (DSCI #4), as described above, developed a Banff IA rejection during an attempted maintenance immunosuppression weaning at post-transplant month 24. None of the control patients had antibody mediated rejection, but one patient (Non-DSCI #1) had a Banff IA BPAR at month 39, which was treated with steroid pulse, resulting in a return to baseline creatinine levels. There was no significant difference in the incidence of BPAR between the DSCI and control groups (P=0.52), and the first BPAR occurred beyond 2 years post-transplant in every instance.

In the DSCI group, 2 patients developed primary disease recurrence at post-transplant month 4 (FSGS) and 17 (MPGN) with return to dialysis at months 37 and 56, respectively. None of the control patients returned to dialysis, and all had good kidney graft function at last follow-up (P=0.17 comparing the 2 groups). Three patients (75%) from the DSCI group developed mild-to-moderate chronic allograft injury demonstrated on protocol and for-cause biopsies 1-4 years post-transplant. Three patients from the control group underwent protocol biopsies (2 patients refused), and mild-to-moderate chronic allograft injury was identified in 2 patients at 1-2 years post-transplant. All DSCI patients had either primary disease recurrence or chronic allograft injury on biopsy. Additionally, 2 DSCI patients (50%) and 1 control patient (20%) developed persistent severe proteinuria (>3gr/24hr) and were placed on angiotensin converting enzyme inhibitors. Neoplasm was not observed in any patients. One study patient (DSCI #2) died at month 47 due to hypertensive encephalopathy after returning to dialysis 37 months post-transplant.

Discussion

Donor bone marrow or stem cell infusion is the most promising approach to date to induce transplant tolerance.[11] Following the first deliberate successful kidney and bone marrow transplantation in a myeloma patient,[2] some transplant centers reported successful tolerance induction to kidney allograft and complete withdrawal of immunosuppression using non-

myeloablative preconditioning (e.g., fractionated total body irradiation, chemical ablation, and/or thymic irradiation, with depleting antibodies) and donor hematopoietic cell transfusion.[12-14] However, because of concerns about toxicities of preparative regimens and post-transplant GVHD, others have tested donor-bone-marrow-infusion protocols without pre-transplant radiation for the induction of tolerance.[3, 7, 15] These better tolerated and less toxic regimens are in line with Monaco's original concept[1] and supported by the discovery of peripheral microchimerism in long-term transplant recipients demonstrating passenger (donor) leukocytes from transplanted grafts.[16, 17] Our previous studies included more than 150 kidney and bone marrow transplant recipients with some immunological benefits, although none was withdrawn from immunosuppression.[5, 18-20]

In this pilot tolerance trial, we utilized C1H for its profound and long-lasting lymphocyte depletion and potential tolerogenic properties.[8-10, 21] Since none of the nine patients experienced a BPAR during the first 2 years post-transplant, C1H as an induction agent to prevent early acute rejection appears effective as previously reported.[22, 23] Nonetheless, the results failed to demonstrate the clinical benefit of DSCI combined with C1H. Unexpected results in the protocol group, such as failed weaning attempts, antibody-mediated rejection with *de novo* DSA, chronic allograft injury, recurrent disease, and proteinuria demonstrate that DSCI combined with C1H induction and weaning immunosuppression were not sufficient to establish transplant tolerance in our study of 4 patients. Although no functional studies were included in this trial, increased frequency of alloreactive memory T-cells after C1H induction may have played a role in the development of an immunological response, and DSCI might have even exacerbated rejection by stimulating residual T-cells escaped from C1H depletion.[24-26] Furthermore, while no significant DSA production was observed in our previous trials[20] and only one patient developed DSA in this trial, it is still possible that DSCI sensitized –instead of regulated– the protocol patients, causing the development of antibody-mediated rejection with *de novo* DSA (DSCI #1) and chronic allograft injury (DSCI #1, #3, and #4).[27] C1H may not be sufficient to prevent antibody-mediated rejection.[28] Our previous trials using a different depleting antibody (i.e., OKT-3) and bone marrow infusion demonstrated that chimerism in the peripheral blood was less than 1% after 6 months post-transplant, while the levels of chimerism in recipient marrow were significantly higher.[29] Unfortunately, detailed chimerism data or mechanistic studies are not available for this trial and the donor cells or sera are not stored for further analyses. Two primary disease recurrences (FSGS, MPGN; 50%) in the treatment group complicated interpretation of data. Since disease recurrence may obscure the development of tolerance induction,[30] future studies aimed at tolerance achievement may be better served by avoiding those primary renal diseases characterized by proteinuria. In conclusion, the above results do not justify extension of this particular trial.

Materials and Methods

With approval from our Institutional Review Board, a pilot, open-label randomized clinical trial was initiated at our center on September 3, 2004. Informed consent was signed by all participating patients.

Patients agreeing to participate were between 18-65 years of age, with body weight >40kg, hematocrit >33%, and normal echocardiogram with ejection fraction >50%. Eligible patients received a primary renal allograft from a non-HLA identical, one haplotype matched living-related donor, with at least 1 HLA DR match and a 1B locus in common, negative B-cell and T-cell cross-match, and PRA <10%. Women were required to have a negative serum pregnancy test. Patients receiving a previous organ transplant were excluded.

Randomization codes were generated by the Statistical Analysis System (SAS) software (SAS Institute, Inc., Cary, NC) and provided to the site in sealed, individual envelopes and were opened sequentially at the time of preparation for transplant. Thus, the assignment of each participant to either treatment (DSCI plus scheduled weaning) or control group was not known until the time of scheduling the patient for transplant (Figure 1b).

A schematic depiction of recipient procedures and treatments is shown in Figure 1a. CD34 + cells were collected from the scheduled DSCI recipients by leukapheresis 30 days before transplant and stored in case of need (e.g., rescue for GVHD). C1H was administered intravenously to all recipients in two doses of 0.3mg/kg, with the first dose given on the day of transplant before kidney revascularization and the second dose four days after transplant. A 500mg intravenous dose of methylprednisolone, 25mg of intravenous diphenhydramine, and 650mg of acetaminophen by mouth were given as premedication before C1H infusion. In both arms maintenance immunosuppression consisted of tacrolimus and MMF. Tacrolimus was given orally twice daily targeting blood trough levels of 5-8ng/ml. MMF was given orally 1gr twice daily, with dose adjusted in response to side effects. Recipients in the protocol treatment group were scheduled to additionally receive DSCI at day 5 and then month 4 post-transplant, immediately prior to planned immunosuppression weaning as described below.

The DSCI study group was subject to a weaning protocol designed as follows: in the absence of BPAR and with graft function being normal (i.e., no noticeable increase in serum creatinine over time), tacrolimus would be discontinued at 4 to 6 months post-transplant after initiation of overlapping therapy with sirolimus, using target trough levels of 8-15ng/ml. After 1 year, sirolimus withdrawal would take place over 6 months providing there was no BPAR or change in serum creatinine. At the completion of year 2, with no BPAR or change in serum creatinine, MMF withdrawal would take place over the next 6 months. Protocol kidney transplant biopsies were scheduled on an annual basis.

Routine postoperative care was provided after the transplant procedure including short-term prophylactic antibiotic treatment. Antiviral prophylaxis with ganciclovir (2.5-5.0mg/kg, IV) was given postoperatively followed by oral valgancyclovir 450-900mg daily for 6 months post-transplant. One single-strength tablet of trimethoprim/sulfamethoxazole was given 3 times a week or dapsone 100mg once a week for Pneumocystis carinii prophylaxis. Nystatin suspension was used for upper gastrointestinal mucosa fungal prophylaxis during the first 3 months post-transplant.

Mild acute rejection (Banff IA) was treated by 3 daily intravenous pulses of 500mg of methylprednisolone, followed by a taper over approximately 2-3 weeks. Higher grades of rejection were treated by Thymoglobulin (1mg/kg per day) for 5-10 days. Intravenous immunoglobulin infusions and/or plasmapheresis and rituximab (375mg/m^2) were administered when there was antibody-mediated rejection diagnosed by development of DSA in recipient sera and C4d staining on biopsy.

All donors were subject to iliac crest puncture for bone marrow harvesting on the day of transplant and leukapheresis procedure at 4 months post-transplant for peripheral CD34+ stem cell harvesting under pegfilgrastim stimulation. Cells were purified from donor marrow obtained from the left iliac crest at surgery by the Isolex® (Nexell Therapeutics, Inc., Irvine, CA) technology for CD34+ stem cells and cryopreserved until re-thawed and administered.

Recipient peripheral blood samples were periodically studied by flow cytometry in order to enumerate percentages and absolute numbers of peripheral immune cell profiles. Kidney biopsy specimens were routinely stained using H&E and trichrome techniques. An immunohistochemistry panel that included C4d staining was also routinely performed.

Grading of BPAR and chronic allograft injury (interstitial fibrosis/tubular atrophy) was performed according to the Banff classification.

Data were presented using the median (range). Statistical comparisons were performed using exact nonparametric methods, e.g., Fisher's exact test for comparing proportions.

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Abbreviations

BPAR	biopsy-proven acute rejection
C1H	alemtuzumab

DSCI	donor stem cell infusion
FSGS	focal segmental glomerulosclerosis
GVHD	graft-versus-host disease
MMF	mycophenolate mofetil
MPGN	membranoproliferative glomerulonephritis

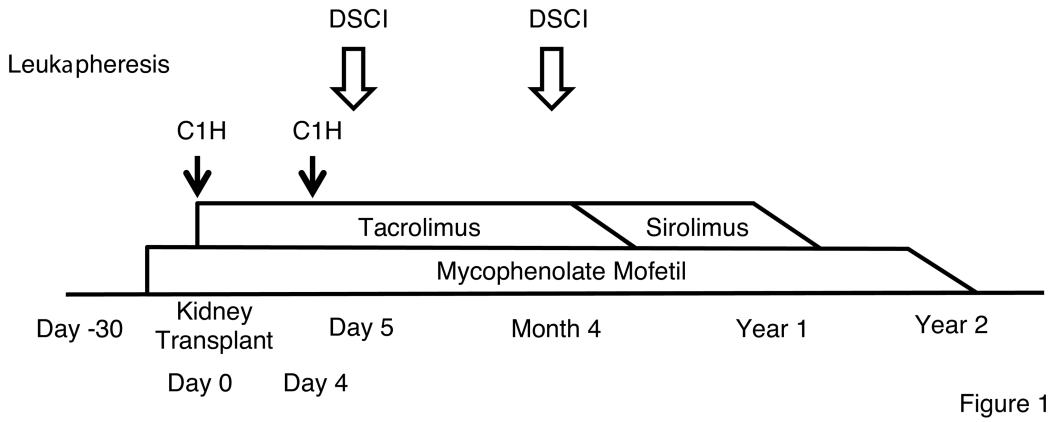

Figure 1a

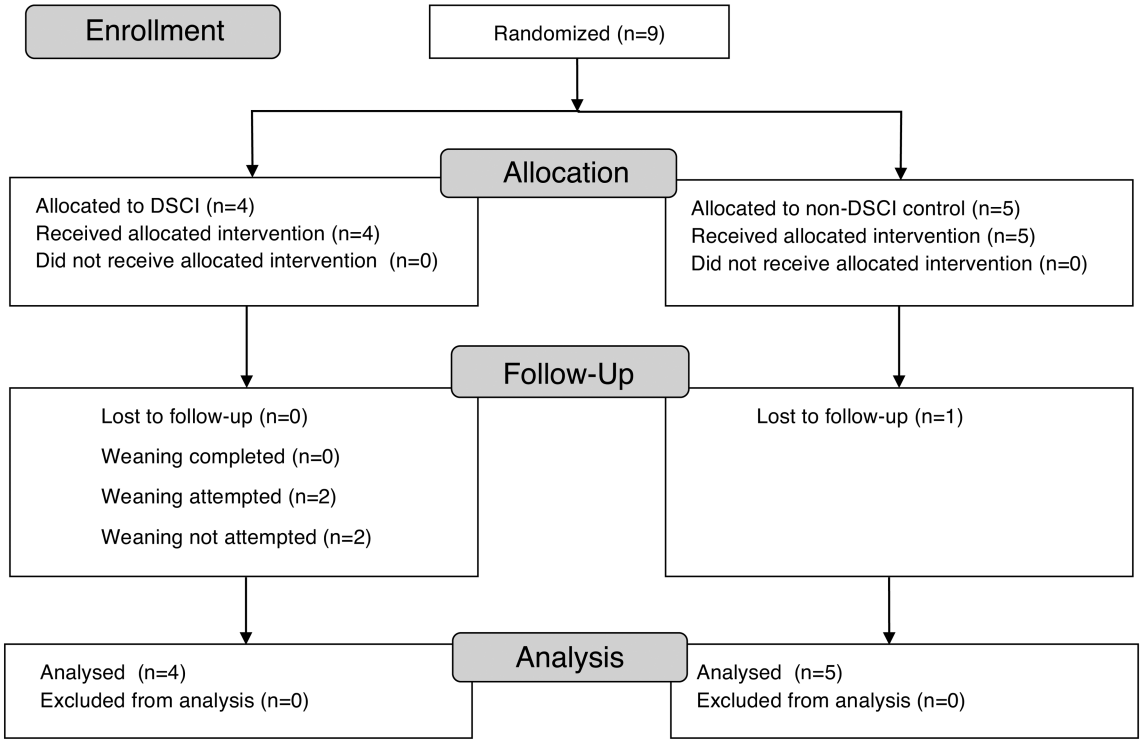

Figure 1b

Figure 1.

a: The design of the treatment protocol. All patients received alemtuzumab (C1H) induction on day 0 and day 4 posttransplant. Study patients received donor stem cell infusion (DSCI) 5 days and 4 months after the transplantation with scheduled maintenance immunosuppression weaning, while control patients did not receive DSCI and remained on the same immunosuppression (tacrolimus and mycophenolate mofetil) without weaning. **b:** Flow diagram of enrolled patients. DSCI: donor stem cell infusion.

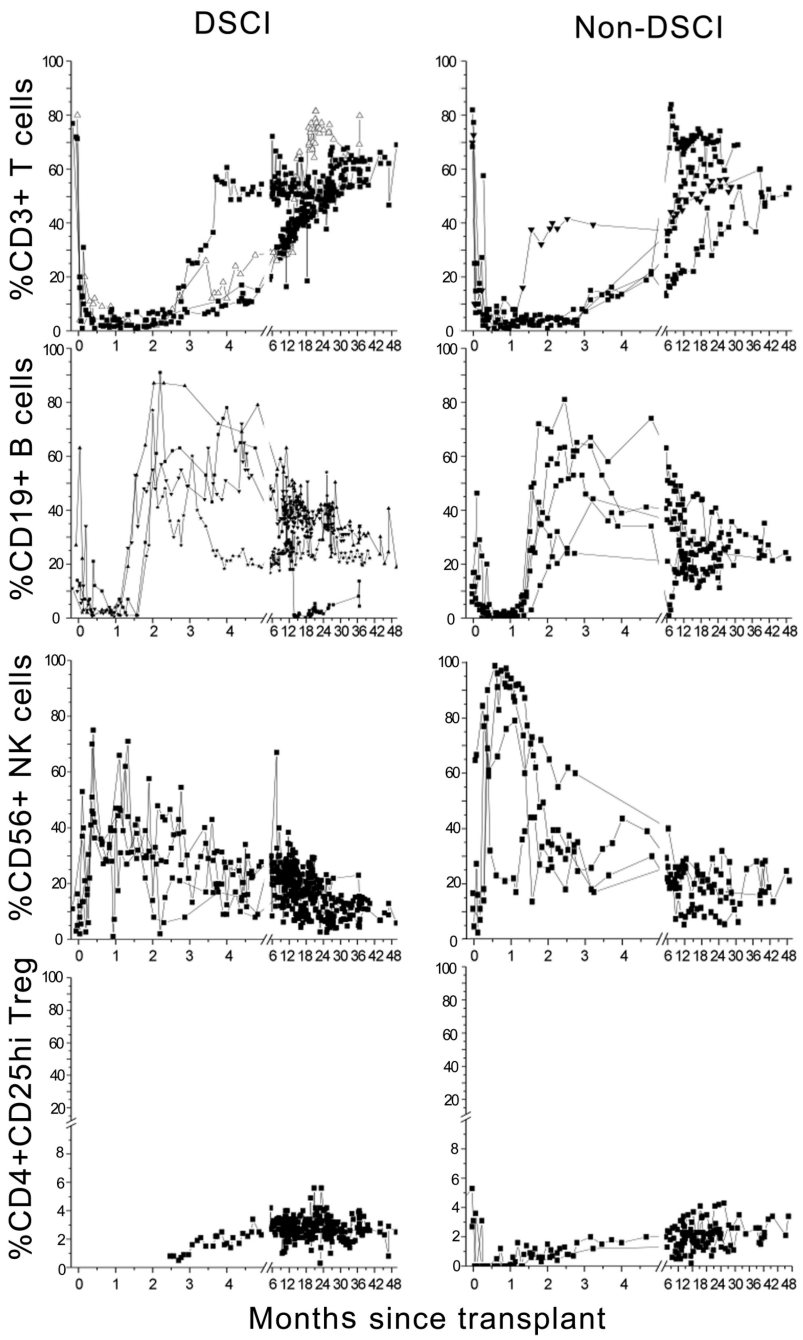

Figure 2.
Peripheral blood lymphocyte profile by flow cytometry in patients who received C1H induction with or without donor stem cell infusion (DSCI). Results are given in percentage.

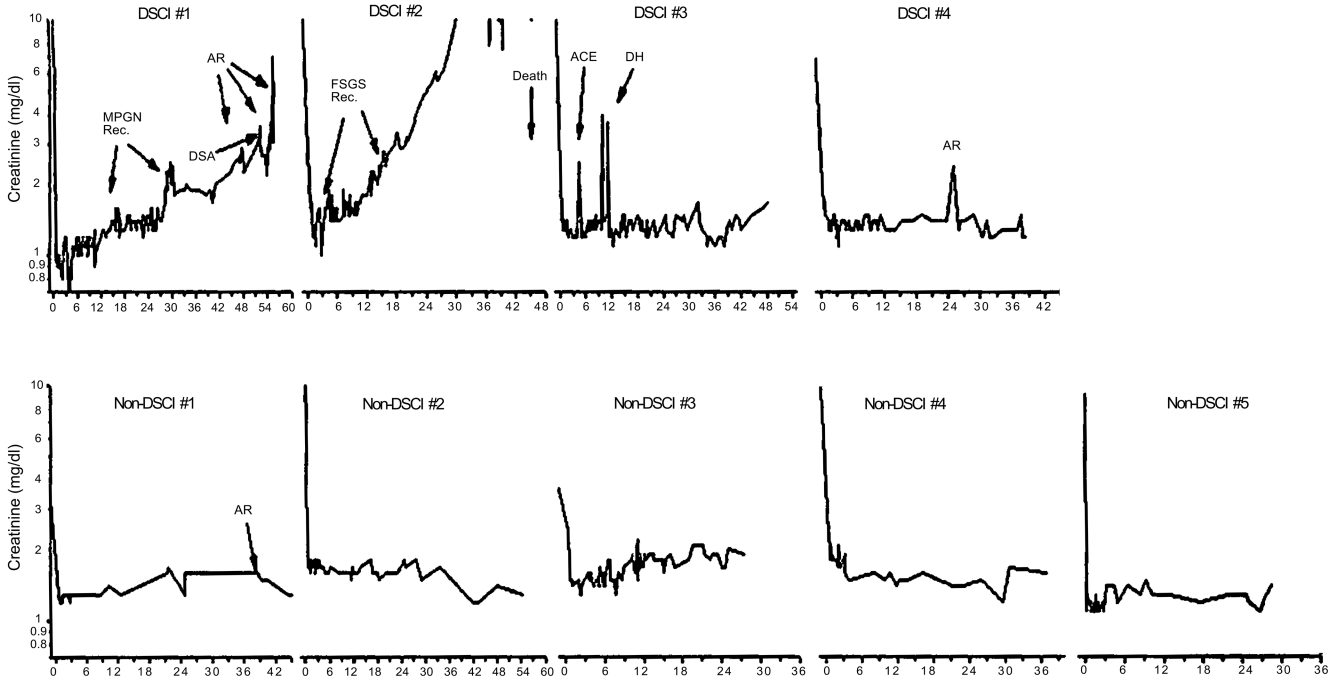

Figure 3.

Evolution of serum creatinine levels and causes of elevations in all patients from the transplant procedure until the last follow up time. Rec.: recurrence of primary disease by biopsy, AR: biopsy proven acute rejection, DSA: donor specific antibody formation, ACE: angiotensin converting enzyme inhibitor toxicity, DH: dehydration.

Table 1

Patient characteristics and overall graft and patient outcome. AMR: antibody mediated rejection, CAI: chronic allograft injury, DSA: donor specific antibody, FSGS: focal segmental glomerulosclerosis, GN: glomerulonephritis, GVHD: graft-versus-host disease, IgA: Immunoglobulin A nephropathy, MGN: Membranous glomerulonephritis, MPGN: membranoproliferative glomerulonephritis, Reflux: reflux nephropathy, TMR: T-cell mediated rejection, Unk: Unknown etiology

Study patients (n=4)					**Control patients (n=5)**				
ID #	DSCI #1	DSCI #2	DSCI #3	DSCI #4	Non-DSCI #1	Non-DSCI #2	Non-DSCI #3	Non-DSCI #4	Non-DSCI #5
Age at transplant	28	19	25	64	40	39	20	18	34
Gender/Race	F/H	F/H	M/H	M/C	M/H	M/C	M/H	M/H	M/H
Primary disease	MPGN	FSGS	Reflux	GN	GN	Unk	Unk	IgA	MGN
Donor relationship	Brother	Mother	Sister	Brother	Sister	Sister	Mother	Mother	Brother
HLA mismatch	1B, 1DR	1A, 1B, 1DR	1B, 1DR	1A, 1DR	1A, 1B, 1DR	1A, 1B	1B, 1DR	1A, 1B, 1DR	1A, 1B, 1DR
Follow up (mo)	61	47	50	43	63	54	32[§]	38	31
Nadir creatinine	0.8	1.0	1.0	1.4	1.1	1.0	1.6	1.2	1.0
TMR	Yes	No	No	Yes	Yes	No	No	No	No
AMR	Yes	No	No	No	No	No	No	No	No
Post-transplant DSA	Yes	No	No	No	No	No	No	No	No
CAI	Yes	No	Yes	Yes	Yes	No	Yes	No	No
Primary disease recurrence	Yes	Yes	No	No	No	No	No	No	No
Proteinuria (>3g/24 hr)	No	Yes	Yes	No	Yes	No	No	No	No
Weaning	No	No	Attempt[*]	Attempt[†]	-	-	-	-	-
Graft loss	Yes	Yes	No	No	No	No	No	No	No
Death	No	Yes	No	No	No	No	No	No	No
GVHD	No	No	No	No	No	No	No	No	No
Severe infection	Yes[‡]	No	No	No	No	No	No	No	No

[*] Tacrolimus and mycophenolate mofetil (MMF) were converted to sirolimus and MMF at month 10; sirolimus was subsequently stopped at month 26 and the patient has been on MMF alone since then. Further weaning was not attempted because of significant proteinuria.

[†] Tacrolimus and MMF were converted to sirolimus and MMF at month 4; sirolimus was subsequently stopped at month 21 and the patient stayed on MMF alone until month 24, when he developed acute rejection. He received steroid bolus and resumption of tacrolimus, MMF and steroids.

[‡] Viral encephalopathy and BK nephropathy

[§] Lost to follow up at 32nd month after transplant.