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Transplantation. Author manuscript; available in PMC 2010 August 27.

Published in final edited form as:
Transplantation. 2009 August 27; 88(4): 533–541. doi:10.1097/TP.0b013e3181b0f92f.

Successful reduction of immunosuppression in older renal transplant recipients who exhibit donor-specific regulation

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Abstract

Background—We hypothesized that T regulatory cells (T_R) specific for donor alloantigens would protect a renal transplant during partial withdrawal of immunosuppression (IS).

Methods—To test this hypothesis, 32 renal transplant recipients >55 years old with excellent renal function were tested for donor-specific regulation (DSR) by trans-vivo delayed type hypersensitivity (TV-DTH) assay at time of enrollment (T=0) and 6 months later (T=6). Twenty-two patients had prednisone withdrawn over a 3 month period, while 10 controls were maintained on triple therapy (prednisone, cyclosporin, mycophenolate).

Results—Out of 22 patients in the steroid withdrawal group, 10 were DSR+, and 12 were DSR- at the time of enrollment (T=0). None of the DSR+ patient experienced acute rejection, nor did any have donor-specific HLA antibody (DSA) during or after withdrawal. Out of 12 DSR- patients, 3 developed acute rejection, which were reversed with bolus steroid treatment, and 4 were DSA + at T=0 or T=6. Two years later, 80% (8/10) of DSR+ patients in the withdrawal group remain steroid free while maintaining excellent renal function, as compared with only 58% (7/12) DSR- patients. Patient survival at 4 years was similar for DSR+ (9/10) and DSR- (11/12) patients in the withdrawal group. Patients maintained on triple therapy remained rejection-free during the 4 yr follow up regardless of initial DSR status, with patient survival rate of 70% (7/10).

Conclusions—DSR prior to steroid withdrawal may identify a subset of transplant patients who could benefit from IS reduction without elevated risk of rejection, or deteriorating renal function.

Introduction

Although the increased number and variety of immunosuppressive (IS) drugs have dramatically improved the short term success of organ transplantation over the past 20 years, two critical problems remain. First, lifelong immunosuppression is associated with significant toxicities including enhanced risk of opportunistic infections and malignancies [especially in older recipients], nephrotoxicity, hypertension and cardiovascular disease (1). Second, medications that have proven successful in the prevention and treatment of acute rejection have had limited success in preventing chronic graft dysfunction and extending long-term graft survival (2). It

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Clinical Trial Registry: MMF Monotherapy and Immune Regulation in Kidney Transplant Recipients: Part 1 Steroid Withdrawal (NCT00214279)

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would therefore be important to exploit natural mechanisms of tolerance to the transplant that could partially or fully replace immunosuppressive drugs, yet provide more effective long-term immunologic protection. Some centers have tried either using initial low-dose therapy, or, after starting with multi-drug immunosuppression, withdrawing one or more of the drugs in clinically well patients at some time after transplantation (1,3,4). These approaches have succeeded in some patients but failed in others (5,6), raising the question: How can one reliably identify candidates for reducing or discontinuing immunosuppression, while avoiding those patients who would be at risk for rejection if similarly reduced?

One approach to identifying low-risk patients is mixed lymphocyte culture (MLC) analysis. This assay has been modified to yield useful information about alloreactivity at a clonal T-cell level (7,8). Yet as a routine screening method it cannot distinguish between responses made by naïve vs. memory T cells, and detects primarily direct pathway alloimmunity, largely missing the low frequency indirect [allopeptide-specific] pathway T cell component that has been shown be critical to tolerance (9). Finally, MLC cannot readily discriminate between potentially damaging and graft-protective alloreactivity. An alternative approach that addresses these concerns is the trans-vivo delayed type hypersensitivity (TV-DTH) assay. The TV- DTH assay is a cell transfer test in which human PBMC are injected into a mouse footpad or ear. When antigen is included, a specific swelling reaction is detectable without exposing the recipient directly to challenge antigens (10,11). Using the footpad transfer method and a SCID or RAG-deficient murine host, TV-DTH can also detect human T regulatory cells (12,13) which cause bystander suppression. Bystander suppression of a DTH recall response in the presence of donor antigen is characteristic of transplant recipients with accepted allografts (11,14). Further investigations have shown that bystander suppression is donor-antigen specific (15), requires both T regulatory and dendritic cells (16) and can be induced by intact donor cells, donor cell lysates, purified HLA-class I antigens (17) or synthetic allopeptides (13). The TV-DTH footpad assay therefore provides a highly sensitive method to probe the indirect pathway of alloreactivity that is critically important both in chronic rejection (18,19) and tolerance (9,20,21). In the present study, we evaluated donor specific regulation (DSR) in renal transplant recipients age 55 years and older enrolled in a steroid withdrawal trial. Our goal was to see if we could retrospectively identify patients with low risk of rejection and donor specific Ab formation.

Materials and Methods

Human subjects

In 2003 the University of Wisconsin transplant program undertook a clinical trial of gradual steroid withdrawal [NCT00214279] in older renal transplant recipients. The over 55 age group was targeted because of the risks of over-immunosuppression therein (22). All blood samples were obtained from patients according to informed consent procedures, subject to human subjects IRB approval at the University of Wisconsin.

Eligibility criteria included: kidney transplant recipients >55 years old, on MMF, Pred, and CNI therapy since the transplant, calculated creatinine clearance >55mL/min, no rejection episode in the past year, stable cardiovascular function, no steroid dependence due to chronic condition (arthritis, gout), HCT ≥ 32mL/dL, and WBC ≥ 3.0 K/μL. African American subjects were excluded due to known high risk of rejection after steroid withdrawal (23).

Trial Design and Power considerations

The original trial design was to enroll 75 patients, age 55 and older. This sample size was calculated to yield 80% power to detect a statistically significant [$p < 0.05$] difference in outcome between patients in the control versus withdrawal group, and/or within the latter group

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based on DSR status. We assumed that approximately 40% of patients with well-functioning grafts [sCr ≤ 1.8 mg/dL], who were > 1 yr post-transplant, would be DSR+ at time of enrollment, based on the demographics and recipient-donor HLA match of the UW-Madison patient population and our previous TV-DTH testing results (24). Furthermore, we assumed an acute rejection event rate of 25% in the non-regulated withdrawal group, and < 5% in the DSR+ withdrawal group. These assumptions proved to be correct; however, due to the difficulty in enrolling older transplant patients that met all the criteria, enrollment lagged and was terminated at n=32 patients.

Patients were randomized at a 2:1 ratio into a steroid withdrawal group and a control group. All patients were maintained on triple drug (PRED, CNI, MMF) therapy at the time of enrollment. Control patients (n=10) remained on triple drug therapy and withdrawal subjects (n=22) underwent a slow taper of steroids over 3 month period to CNI and MMF.

A minimum of two TV-DTH assays were performed on each patient: first, at the time of enrollment (T=0) and second, 6 months later (T=6) after completion of gradual steroid withdrawal. In some patients, a third sample was obtained at 8–9 months after enrollment, on suspicion of acute rejection. One patient who had a rejection episode prior to completing steroid withdrawal was tested at T=2 months time point.

To determine if there were any long-range benefits or adverse consequences of reduced immunosuppression, patients were followed-up for renal function over a 4 yr period.

It should be emphasized that neither randomization into withdrawal vs. control group, nor any clinical decisions regarding patient care, were made based on TV-DTH assay results. It should also be noted that DSA testing was performed on stored serum/plasma samples well after completion of the trial.

Peripheral blood mononuclear cells (PBMC) isolation

PBMC were obtained by sterile venepuncture and collected into ACD tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) and further purified by Ficoll-density centrifugation (Cellgro; Mediatech, Inc., Herndon, VA, USA) according to company protocol. PBMC were washed 3–4 times in Dulbecco's phosphate-buffered saline (Cellgro; Mediatech, Inc.) to reduce platelet contamination.

Mice

CB-17 SCID mice were bred at the University of Wisconsin Gnotobiotic Laboratory facility. All animals were housed and treated in accordance with guidelines outlined by the University of Wisconsin and the National Institutes of Health.

Trans- vivo DTH analysis

We injected 7–9×10^6 PBMC, along with donor antigen into the footpad of 6–8 week old SCID mice, as described previously (24). The response to inactivated Epstein-Barr virus (EBV) or tetanus toxoid (TT) recall antigen alone plus PBMC was used as a positive control, with PBMC + PBS as a negative control. To test for bystander suppression, a recall antigen, was co-injected with donor antigen. Antigen-driven swelling was determined as previously described (11). DTH reactivity is shown as the change in footpad thickness in multiples of 10^{-4} inches, measured using a dial thickness gauge [Mitutoyo, Japan].

The extent of bystander suppression was measured as % inhibition of recall antigen response in the presence of donor antigen, calculated using the following formula:

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$$\% \text{ inhibition} = 1 - [(\text{Recall+donor Ag})/(\text{Recall})] \times 100\%$$

as previously described (16). Donor-specific regulation (DSR+) is characterized by low anti-donor response ($<25\times10^{-4}$ in.) and at least 50% inhibition of the recall Ag response in the presence of donor Ag based on studies of transplant tolerance in patients, monkeys, and mice (11,13,14,25,26). Donor-specific non-regulator status (DSR-) was designated as an inhibition of < 50%.

The DSR- group encompassed both "non-responder" (NR) and "sensitized" (S) patients, based on anti-donor antigen response of < or $\geq25\times10^{-4}$ in., a value which represents a minimum positive control response to recall antigen in TT- or EBV-immune subjects.

Source of antigen

Donor antigen was prepared from either fresh PBMC or frozen splenocytes as described previously (10,11). EBV antigen was purchased from Viral Antigens, Inc. (Memphis, TN, USA), TT antigen was manufactured by Wyeth-Ayerst Pharmaceuticals (Pearl River, NY, USA).

Anti-HLA Ab detection

Stored samples of serum/plasma collected at the time of enrollment [T=0] and 6 mos. later [T=6] were tested in blinded fashion at a 1:3 dilution for anti-HLA Ab by indirect immunofluorescence using the PRA class I and class II mixed antigen screening system to evaluate reactivity pattern (LabScreen mixed antigen beads; One Lambda Inc.). Single HLA-coated beads were used as a secondary screen to definitively identify DSA.

Positive and negative cutoff values were determined based on specific immunofluorescence values for a given batch of HLA-coated beads, and on the ratio of sample MFI to negative control MFI ≥2 for a positive response. All patients underwent routine pre-transplant screening by standard T cell cross-match techniques in the pre-Luminex era.

Statistical analysis

To test for differences between the control and withdrawal groups, Fisher's exact tests were used for categorical variables [such as DSR $\geq50\%$ vs. DSR $<50\%$], and Kruskal-Wallis tests for continuous variables. HLA match variables (A, B, DR and DR alone) were treated as continuous variables. We used a Kaplan-Meier analysis and log rank test to test the impact of DSR at T=0 on freedom from acute rejection after steroid withdrawal. As a secondary variable besides DSR, we also looked at donor-TV-DTH reactivity [sensitized phenotype]. $P<0.05$ was used as the criterion for statistical significance.

Results

Table 1 shows the demographics of the 32 primary kidney transplant recipients enrolled in this trial. At the time of enrollment and, all patients had stable graft function. There were no significant differences between the control and withdrawal groups, except that enrollment was somewhat later after transplant for the control group as compared with the withdrawal group (p=0.015).

Figure 1 illustrates the DSR+ and DSR-phenotypes detected by the TV-DTH assay. Patient DD5 exemplifies the DSR+ pattern, characterized by a weak response to donor antigen (10×10^{-4} in.) and a marked bystander suppression (60% inhibition) of recall antigen response

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in the presence of dAg at T=0. 6 months later, the same pattern of bystander suppression was found. A renal biopsy performed at month 9 showed no evidence of acute rejection (A0) and the patient has remained steroid-free with excellent renal function [sCr = 1.4 mg/dL] at 4 years. In contrast, patient LURD 6 exemplifies the non-regulator DSR- pattern. At T=0 patient exhibits a non-responder (NR) phenotype, which had the feature of a weak response to donor (Δ10) and a low bystander suppression score (29% inhibition). Six months later the patient exhibits a donor- sensitized (S) pattern, characterized by a high response to donor antigen (27.5×10^{-4} in.) with no bystander suppression (0%). A biopsy performed 2 months later confirmed suspicion of rejection (A2 score) and the patient was returned to triple therapy [Fig. 1B].

Table 2 summarizes TV-DTH results and clinical outcomes in the DSR+ and DSR- steroid withdrawal group of patients. We found a wide range of bystander suppression values in T=0 PBMC, from 0–100% inhibition of recall antigen response. Of the 22 steroid withdrawal subjects, 10 were DSR+ and 12 were DSR- at the time of enrollment (T=0). None of the DSR + patients became sensitized to donor antigen in TV-DTH, nor did any developed donor specific antibodies (DSA) at either time point. Of the 10 DSR+ subjects at T=0, 8 were still DSR+ 6 months later, while 2 (DD 8, LRD 11) became DSR- (NR); all were successfully withdrawn from steroids with no cases of acute rejection or DSA formation.

In the group of 12 DSR- patients at T=0, all remained DSR- at T=6; 9/12 were successfully withdrawn from steroids. Two of these 9 patients (DD 3 and DD 10) had a positive anti-donor TV-DTH response (sensitized phenotype) at T=0 but lost this response at T=6; one other, patient DD 9, became DTH-sensitized (S) to his donor at T=6. However, DD3, DD10, and DD9 developed neither DSA nor rejection. The remaining 3 DSR- patients (LURD 6, LURD18, LURD 14) all experienced an acute rejection episode necessitating return to triple drug IS.

Figure 2 shows a Kaplan –Meier plot of biopsy-proven acute rejection episodes in the steroid withdrawal group. Although the sample size [n=22] was too low to detect a significant difference between DSR+ vs. DSR- patients (p=0.0977 by log rank test), it should be noted that 25% (3/12) DSR- patients had an acute rejection within the first 8 months after enrollment. One patient (LURD 18) started withdrawal with 2 donor-specific class II Abs (anti-DR17 and –DQ2), and began to reject at 2 months, before the 3-months steroid withdrawal process was completed. This patient had developed donor antigen reactivity (25×10^{-4}in.) by TV-DTH, and de novo DSA (to DR10) at the time of rejection. The biopsy showed acute cellular rejection and C4d deposition. A second patient, LURD 6, completed the withdrawal but showed a dramatic increase in anti-donor response at 6 months (Fig. 1B). Two months later he developed biopsy-proven A2 cellular rejection, as well as humoral rejection by C4d deposition. A third DSR- patient, LURD 14, completed the steroid withdrawal protocol and remained a non-responder to donor antigen at T=6; however, due to cyclosporine nephrotoxicity, he was returned to corticosteroid plus MMF dual therapy and cyclosporine was abruptly withdrawn. LURD 14 was found to be DTH-sensitized at 8 months. The renal transplant biopsy revealed a low grade A1 cellular rejection and positive C4d staining. However, unlike DSA+ patients LURD 6 and LURD 18, we were unable to detect any donor-specific anti-A, B, DR or DQ antibody at the time of a biopsy proven rejection in patient LURD 14.

The long-term success of steroid withdrawal is shown in Table 2 [last column], and in Figure 3A. Overall, 80% (8/10) of DSR+, but only 58% (7/12) of DSR- patients were still steroid-free at 2 years post-transplant, mainly due the higher rejection rate in the latter group. In addition to the 3 patients in the DSR- steroid withdrawal group that were returned to triple IS therapy due to rejection, an additional 4 patients in the withdrawal arm were re-started on prednisone or dexamethasone: 2 DSR+ patients due to severe polyarticular gout (DD 2) and arthralgias (DD 13), and 2 DSR- patients due to Addison’s desease (DD 10), and myalgias (LRD 9).

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As shown in Figure 3A, renal function was uniformly excellent in the DSR + withdrawal patients over a follow-up period of 4 years. The only DSR+ patient who did not survive to year 4 after steroid withdrawal was DD12 (* on Fig. 3A). The only re-transplant recipient in the study, he became non-compliant after 24 months, with evidence of excessive alcohol use, and died with a functioning graft at 33 months. Renal function at 4 years in the remaining 9 DSR + patients fell within the pre-enrollment range (0.9–1.7 mg/dL).

In the DSR- withdrawal group, 8/12 maintained excellent renal function at 42 months (range 0.8–1.4 mg/dL), but 4 had sCr values exceeding 1.7 mg/dL. Of these, two had already experienced an acute rejection episode (LURD 14 and LURD 18), and one (DD 10) died at 44 mos. due to renal complications arising from an angiography procedure. The fourth (LURD 4; sCr= 2.3mg/dL at 48 mo.) has remained steroid-free, with a current serum Cr of 1.7 mg/dL.

Table 3 shows a summary of TV-DTH results in the control group. Of the 10 patients in this group, 3 were DSR+ and 7 were DSR- at T=0. None of the patient experienced any episodes of allograft rejection, regardless of DSR status. All 3 DSR+ patients were DSA-, while 3/7 DSR- patients (DD 7, LURD 5, LURD 16) had donor specific antibodies at one or two time points, consistent with observations in the withdrawal group. As shown in Figure 3B, all 10 patients in the control group maintained sCr in the range of 0.9–2.0 mg/dL at 12 months post-enrollment and the 7 still surviving at 4 years had similar excellent renal function. Three patients (30%) died with functioning grafts [* on Fig. 3B].

Considering all 32 patients enrolled, 7/19 DSR- patients, and 0/13 DSR+ patients had DSA at one or both time points. The difference in DSA formation between DSR+ and DSR - patients was highly significant [$p < 0.025$ by Fisher's exact test]. In addition, the difference in HLA-DR match between DSR+ (1.31 ± 0.48; n=13) and DSR- patients (0.53 ± 0.61; n=19) was also highly significant (p=0.003 by Wilcoxon test).

Discussion

In the present study we tested the hypothesis that the presence of circulating donor antigen-specific T regulatory cells, as indicated by bystander suppression of TV-DTH, predicts safety of steroid withdrawal in older renal transplant recipients. Our results tend to support this hypothesis, as none of the DSR+ patients developed a donor-specific alloantibody [p=0.025 vs DSR-; n= 32] or experienced acute rejection episodes after steroid withdrawal [p=0.097 vs. DSR-; n=22]. The overall success rate for steroid withdrawal was higher in the DSR+ group: 8/10 [80%] DSR+ patients remained steroid free, without compromising their long-term kidney function, compared to only 7/12 (58%) in DSR- group. This suggests the DSR+ phenotype is a prognostic indicator for success of partial withdrawal of IS in this patient population.

The DSR- group included a majority of patients (8/12) that underwent successful steroid weaning without developing DSA or rejection. This result suggests that at least some older patients who lack allo-specific T regulatory cells may nonetheless have a low enough frequency or functionality of alloreactive T and B cells that the presence of 2 drugs only [Cyclosporine and MMF] is still sufficient to protect the graft. We do not know whether by further reducing IS drugs [for ex., to MMF monotherapy or no IS] such a DSR- graft recipient might begin to suffer from uncontrolled effector responses, as has been observed in Rhesus monkey (14) and human renal transplants (11–13,27) on minimal or no immunosuppression.

The metastable nature of peripheral tolerance in the early post-transplant period, originally proposed in mice by Lafferty (28)has been confirmed in monkey and human renal transplantation (12–14) and was evident in the current study. While loss of regulation may predispose to graft rejection after complete IS withdrawal, 2/10 withdrawal group patients who were DSR+ at T=0 had lost regulation 6 months later, yet this fluctuation did not appear to

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result in any adverse consequences. Similarly, transient anti- donor reactivity at T=0 in 2/12 DSR- withdrawal group patients that disappeared by T=6, did not result in rejection during or after steroid withdrawal. Thus an initially "DTH-sensitized" phenotype was not predictive of outcome, as has been previously reported (29). One critical co-factor may be prior or concurrent antibody formation (27), as in pts LURD 6 & 18 who became DTH-sensitized after steroid reduction and experienced rejection episodes.

While prior DSA testing could have excluded 2/3 patients that developed acute rejection after steroid withdrawal, the exception, LURD 14 who had a rejection after cyclosporine withdrawal and steroid re-start at T=6, indicates that DSA status alone may not be sufficient criteria to rule out a given candidate for reduction of immunosuppression. Rather, a combination of DSA [B cell] and DSR [T cell] analysis may define a hierarchy of risk after IS reduction. The significant negative correlation of DSR with DSA [p=0.025 by Fisher's exact test of n=32 pts] has been recently confirmed in a Campath-1H induction/sirolimus monotherapy trial (30) and suggests interference of donor-specific T reg cells with a) indirect pathway T help needed for alloantibody formation, or b) B cells entering germinal centers (31).

Several investigators (32,33) have shown a relationship between HLA match and graft survival, with HLA-DR matching having the greatest beneficial effect on graft outcome in kidney transplantation (34). A strong correlation between DSR and the degree of HLA- DR matching, reported previously by our group (24), was confirmed in the present study.

In contrast to our findings, and to DTH transfer studies in mouse transplant models (25,35) Pelletier et al. (36) using only immunocompetent mice and ear injection site for PBMC injection, found that detection of bystander suppression response to donor antigens does not identify patients that have developed graft protective, regulatory T-cell responses. It should be noted that in this study there were several differences in the TV-DTH assay method from that used in our study. While both ear and footpad TV-DTH methods can readily detect effector responses, PBMC from tolerant transplant recipients mediated bystander suppression only when human cell injections were made into the footpads, and not ears, and only in immunodeficient, not immunocompetent mice (37). The reasons for this are still not entirely clear; however, studies currently underway in our lab indicate that transferred human cells are better retained in the footpad than in the ear (38).

The tremendous advantages of the TV-DTH assay in the SCID mouse footpad include: 1) that it detects both Th-1 and Th-17 effector responses (39,40), 2) that it detects both IL-10 and TGF-β dependent regulatory T cells (12,13,26), and 3) it has the added convenience that crude cell lysates can be used to detect DSR. Nonetheless, there are drawbacks inherent in the TV-DTH test that limit its widespread clinical use: 1) the requirement for a mouse as adoptive host limits the assay to research labs with available animal facilities; 2) the use of the footpad raises animal welfare concerns in certain countries; and 3) the technique itself requires extensive training, particularly in the bystander suppression assay. Thus we believe that TV-DTH is a first-generation assay for monitoring DSR. The recent finding by Derks, et al. (16) that dendritic cell products released in response to donor antigen-triggered T_R cells are critical for bystander suppression, suggests that second-generation DSR assays may include analysis of DC– derived thrombospondin-1 [TSP-1] and indoleamine-2,3 dioxygenase [IDO], or detection of upstream elements of the extracellular ATP signaling pathway required for TSP-1/IDO synthesis and release (16,41).

In summary, our results in this small clinical trial, while not definitive, strongly suggest that donor-specific regulation in patients >55 years old with stable kidney graft function, is a favorable prognostic indicator for reduction of IS therapy, an otherwise risky undertaking (42). Our findings warrant further prospective studies of DSR screening in settings of post-

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transplant IS reduction trials, including trials of calcineurin inhibitior weaning such as the MMF monotherapy trial in living-related kidney transplant recipients currently underway at our center.

Acknowledgments

This work was supported by grants from National Institutes of Health 1R21-DK077354-01by an investigator-initiated grant from Roche Pharmaceuticals and by research grant #595231414 from ROTRF. The authors would like to thank N. Radke, C. Lillesand, C. Janus, and M. Schmidt for their efforts at patient recruitment. We also thank Keith Smart for his assistance.

Abbreviations List

TV-DTH
: trans-vivo delayed type hypersensitivity

DSR
: donor specific regulation

DSA
: donor specific antibody

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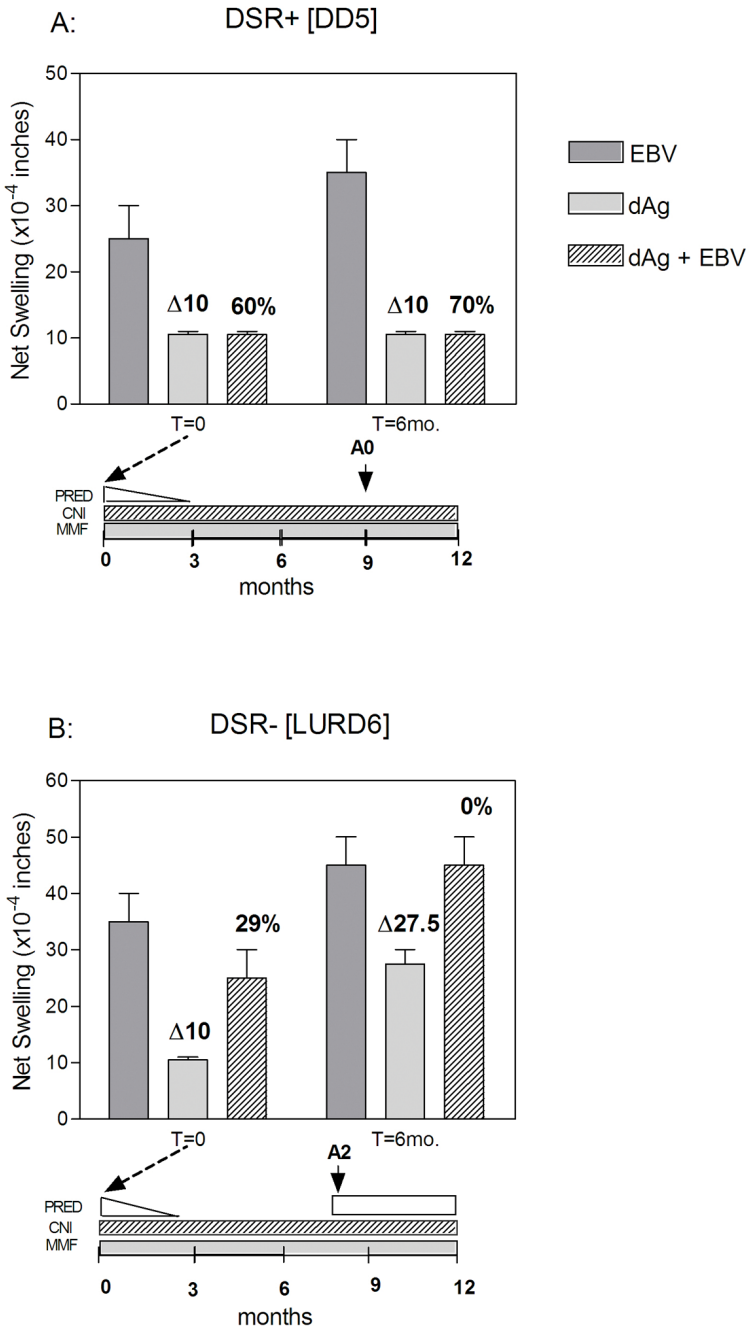

Figure 1.

TV-DTH assay results from two selected patients exemplifying the DSR+ (A) and DSR- (B) phenotypes. TV-DTH testing was performed at T=0 and T=6 mo. For TV-DTH assay 7×10^6 PBMCs from each patient were mixed in different combination with dAg or EBV, or combination of both and injected into the footpads of SCID mice. Net swelling response was measured after 24 hours. Background swelling (PBMC+PBS) varied from $10–30\times10^{-4}$ in. and was subtracted from raw values to calculate the net swelling response to each antigen or antigen combination; % inhibition values were determined as described in Methods. Bars indicate mean ± SEM for duplicate assays. Diagram below each figure shows the time course of IS drug

therapy in each patient. Dashed arrow indicates T=0 the beginning of steroid withdrawal process. (↓, A0–2) indicates biopsy time point and score.

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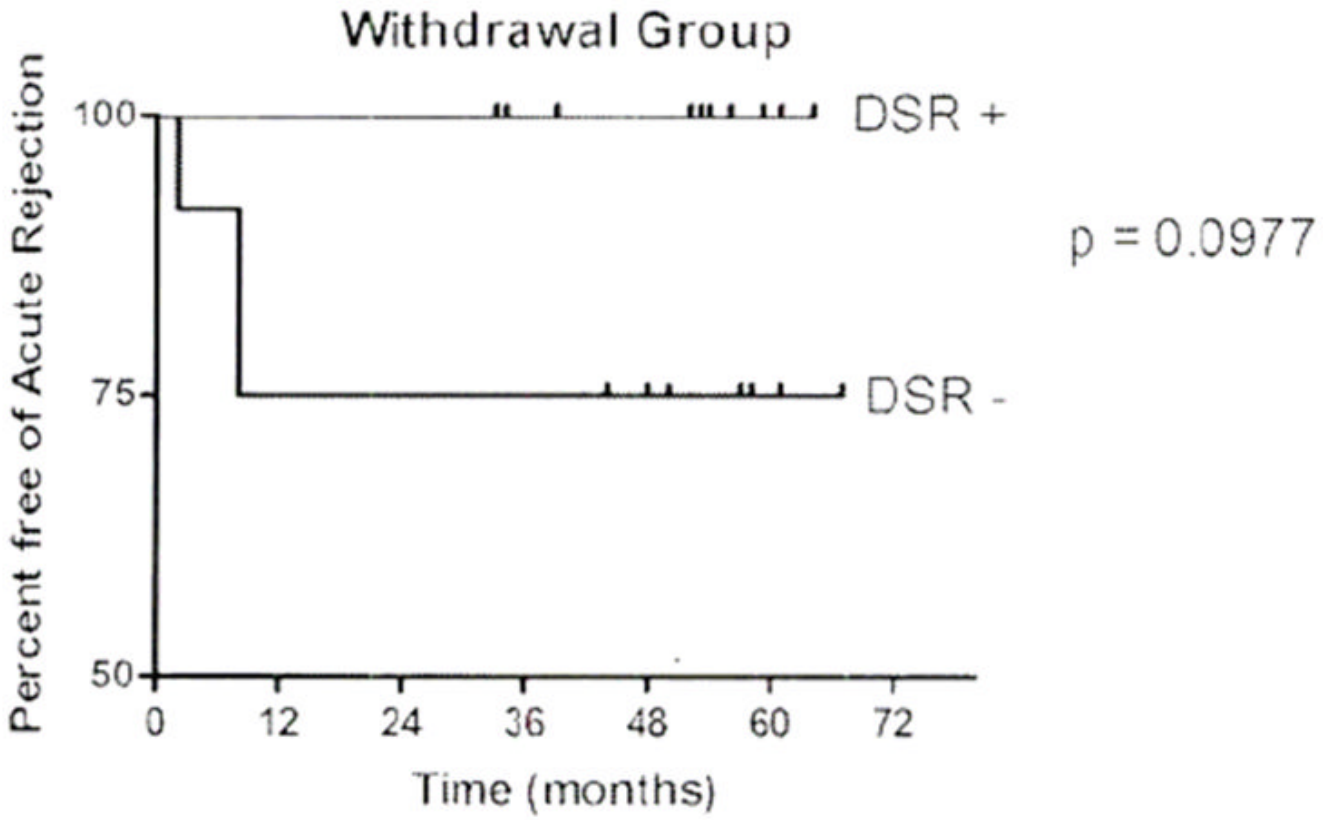

Figure 2.
Kaplan-Meier plot showing incidence and timing of acute rejection episodes in the withdrawal group [n=22 pts]. Log rank p value comparing DSR+ and DSR- pts is shown.

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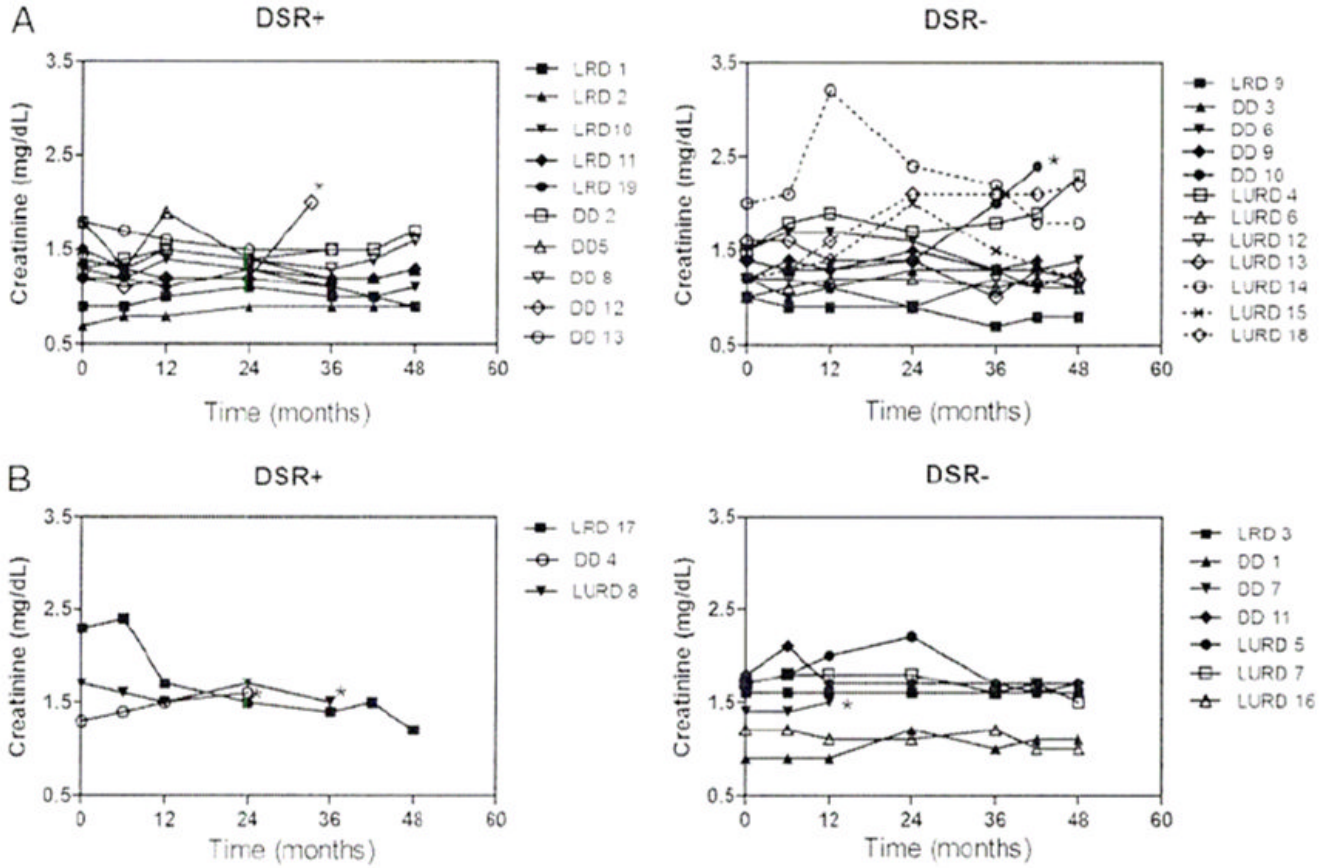

Figure 3. Serum creatinine values over a 4-year period

A: Withdrawal group. Patients with initial DSR + status are shown at left; those with initial DSR- status at time T =0 [0 time point on x-axis] are shown at right. Dashed lines indicate patients in withdrawal group who had an acute rejection episode and were returned to triple drug IS.*Two patients died with functioning grafts: DSR+ patient DD 12 due to unknown causes at 33 mos., after excessive alcohol abuse and non-compliance with all medication at 24 mos.; and DSR- pt DD10 as a result of renal complications from an angiography procedure. B: Control group. Patients with initial DSR score ≥ 50% (DSR+) are shown at left; those with initial DSR score < 50% (DSR-) at time T =0 [0 time point on x-axis] are shown at right. (*) Indicates patients who died with functioning grafts: DSR+ patient DD 4 due to malignant disease, DSR+ LURD 8 due to myocardial infarction, and DSR-patient DD 7 who died in an car accident.

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Table 1

Demographics of patients in DTH study

Category	Control group n or mean ± SD	Withdrawal group n or mean ± SD	Significance p value
Number of patients	10	22	
Renal transplant from:			
1-Haplo LRD[a]	2	6	
DD	4	9	1
LURD	4	7	
Age at Tx	55.73 ± 3.23	59.10 ± 4.55	0.095
Years post-Tx at enrollment[b]	5.43 ± 2.79	3.49 ±2.28	0.015
Gender (M/F)	9/1	14/8	0.21
Creatinine (mg/dL) at T=0	1.56 ± 0.38	1.33 ± 0.31	0.097
HLA A, B, DR match:	2.10 ±2.08	2.45 ± 1.37	0.492
HLA DR match:	0.80 ± 0.79	0.86 ±0.64	0.753
Therapy:			
T=0	Triple[c]	Triple	
T=6 mo.	Triple	Dual[d]	
DWF[e]	3	2	0.293

[a] abbreviations: 1 HLA haplotype identical living related donor (1-Haplo LRD), deceased donor (DD), living unrelated donor (LURD)

[b] enrollment is T=0; all patients were tested in TV-DTH at T=0

[c] prednisone, mycophenolate, calcineurin inhibitor (cyclosporin or tacrolimus)

[d] mycophenolate, calcineurin inhibitor e- death with functioning graft

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Table 2

Summary of TV-DTH results in steroid withdrawal group.

DSR + (inhibition score ≥ 50%)

Withdrawal	DR Match	Inhibition (%) T=0[a]	Donor Reactivity at T=0		Inhibition (%) T=6 mo.	Donor Reactivity at T=6mo.		Rejection	Prednisone at 24 mo.
			TV-DTH[b]	DSA		TV-DTH	DSA		
LRD 1	1	100	-	-	50	-	-	-	-
DD 8	2	70	-	-	33*	-	-	-	-
DD 12	1	67	-	-	70	-	-	-	-
LRD 2	1	60	-	-	67	-	-	-	-
LRD 11	1	60	-	-	12*	-	-	-	-
DD 5	2	60	-	-	70	-	-	-	-
DD 13	1	60	-	NT	60	-	-	-	+
LRD 19	1	57	-	-	60	-	-	-	-
LRD 10	1	50	-	-	50	-	-	-	-
DD 2	2	50	-	-	83	-	-	-	+
n=10	1.30±0.48	63.4±14.31	0/10	0/9	55.5±20.57	0/10	0/10	0/10	8/10 (80%) off

DSR - (inhibition score < 50%)

Withdrawal	DR Match	Inhibition (%) T=0[a]	Donor Reactivity at T=0		Inhibition (%) T=6 mo.	Donor Reactivity at T=6mo.		Rejection	Prednisone at 24 mo.
			TV-DTH[b]	DSA		TV-DTH	DSA		
DD 3	1	43 (S)	30	-	25	-	-	-	-
LURD 13	0	40	-	-	25	-	DQ2	-	-
DD 9	1	30	-	-	0 (S)	30	-	-	-
LURD 6	0	29	-	DQ6	0 (S)	27.5	DQ6	8 mo.	+
LURD 4	0	22	-	DQ2	0	-	DQ2	-	-
DD 10	0	20 (S)	25	-	33	-	-	-	+
LRD 9	1	17	-	-	17	-	-	-	+
DD 6	1	0	-	-	40	-	-	-	-
LURD 12	1	0	-	-	0	-	-	-	-
LURD 14	0	0	-	-	25	-; 25[c]	-	8 mo.	+
LURD 15	1	0	-	-	33	-	-	-	-
LURD 18	0	0	-	DR17, DQ2	0(S)[d]	25[d]	DR10,17; DQ2	2 mo.	+
n=12	0.50±0.52	16.8±16.50	2/12	3/12	16.5±15.59	3/12	4/12	3/12	7/12 (58%) off

[a] All patients are “regulator” (R) phenotype at T=0

[b] TV-DTH responses <25×10^{-4} inches (−)

* Patients changed to a “nonregulator” (NR) phenotype at T=6

[a] All patients are “non-regulator” (NR) or sensitized (S) phenotype at T=0; the latter patients at each time point are indicated by (S).

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[b] TV DTH responses $<25\times10^{-4}$ inches (−)

[c] time of rejection T=8 mo.;patient was switched from CyA/MMF to Pred/MMF at T=6 due to cyclosporin nephrotoxicity;returned to triple therapy at 8 mo. due to rejection

[d] at the time of rejection T=2 mo.

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Table 3

Summary of TV-DTH results in control triple IS group

DSR + (inhibition score ≥ 50%

Control	DR Match	Inhibition (%) T=0[a]	Donor Reactivity at T=0		Inhibition (%) T=6mo.	Donor Reactivity at T=6mo.		Rejection	Prednisone at 24 mo.
			TV-DTH[b]	DSA		TV-DTH	DSA		
LRD 17	1	67	-	-	67	-	-	-	+
DD 4	2	50	-	-	14 (S)[c]	30	-	-	+
LURD 8	1	50	-	-	67	-	-	-	+
n=3	1.30±0.58	55.7±9.81	0/3	0/3	49.3±30.6	1/3	0/3	0/3	3/3 on

DSR - (inhibition score < 50%)

Control	DR Match	Inhibition (%) T=0[a]	Donor Reactivity at T=0		Inhibition (%) T=6mo.	Donor Reactivity at T=6mo.		Rejection	Prednisone at 24 mo.
			TV-DTH[b]	DSA		TV-DTH	DSA		
LURD 5	0	43 (S)	25	A1	25	-	-	-	+
DD 7	0	38	-	-	0	-	DQ2	-	+[*]
LURD 7	0	37	-	-	17	-	-	-	+
DD 1	1	33	-	-	20 (S)	25	-	-	+
LURD 16	0	20	-	B35	33	-	B35	-	+
LRD 3	1	0	-	-	33	-	-	-	+
DD 11	2	0 (S)	35	-	33	-	-	-	+
n=7	0.57±0.79	24.4±18.14	2/7	2/7	23.0±12.10	1/7	2/7	0/7	7/7 on

[a] All patients are “regulator” (R) phenotype at T=0

[b] TV-DTH responses <25×10^{-4} inches (−)

[c] patient with sensitized phenotype (S) at T=6mo.

[a] All patients are “non-regulator” (NR) or sensitized (S) phenotype at T=0; the latter patients at each time point are indicated by (S)

[b] TV-DTH responses <25×10^{-4} inches (−)

[*] Deceased at18 mo. while on steroids (see Fig 3b)