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Living donor kidney transplantation in crossmatch-positive patients enabled by peritransplant immunoadsorption and anti-CD20 therapy

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Summary

Living donor kidney transplantation in crossmatch-positive patients is a challenge that requires specific measures. Ten patients with positive crossmatch results ($n = 9$) or negative crossmatch results but strong donor-specific antibodies (DSA; $n = 1$) were desensitized using immunoadsorption (IA) and anti-CD20 antibody induction. IA was continued after transplantation and accompanied by HLA antibody monitoring and protocol biopsies. After a median of 10 IA treatments, all patients were desensitized successfully and transplanted. Median levels of mean fluorescence intensity (MFI) of Luminex-DSA before desensitization were 6203 and decreased after desensitization and immediately before transplantation to 891. Patients received a median of seven post-transplant IA treatments. At last visit, after a median follow-up of 19 months, 9 of 10 patients had a functioning allograft and a median Luminex-DSA of 149 MFI; serum creatinine was 1.6 mg/dl, and protein to creatinine ratio 0.1. Reversible acute antibody-mediated rejection was diagnosed in three patients. One allograft was lost after the second post-transplant year in a patient with catastrophic antiphospholipid syndrome. We describe a treatment algorithm for desensitization of living donor kidney transplant recipients that allows the rapid elimination of DSA with a low rate of side effects and results in good graft outcome.

Introduction

Successful living donor kidney transplantation in patients with a positive crossmatch and donor-specific antibodies (DSA) requires specific measures, such as plasmapheresis or intravenous immunoglobulins [1,2]. Despite these measures, impaired graft survival rates were reported in desensitized living donor kidney transplant recipients [3–7]. Recently, our group documented in a small series of living donor kidney transplant recipients that repeated immunoadsorption (IA) in combination with anti-CD20 therapy is efficient in removing pretransplant DSA [8]. In this series of transplants, IA was accomplished by two parallel, regenerable columns that have the peptide peptide-GAM (Globaffin) covalently bound to sepharose. The Globaffin columns bind IgG subclasses 1, 2 and 4 with high affinity and IgG3, IgA and IgM with variable affinity [9,10]. During one IA treatment, 87% of IgG may be eliminated from the systemic circulation, whereas albumin or antithrombin III remains almost unaffected. With multiple IA treatments, more than 98% of IgG or a specific antibody such as DSA may be eliminated from the systemic circulation without a need for substitution with fresh frozen plasma or albumin [11,12]. Moreover,

compared with plasmapheresis, IA is associated with better tolerability and a lower likelihood of allergic reactions, and allows therefore the treatment of larger plasma volumes in individual patients with higher antibody reduction rates [12,13].

Herein, we present data on a consecutive series of 10 living donor kidney transplant recipients who had a positive complement-dependent cytotoxicity (CDC) and ELISA crossmatch ($n = 9$) or a negative crossmatch result but strong DSA in ELISA and Luminex testing against the donor ($n = 1$) and who were transplanted following desensitization by IA.

Patients and methods

Apheresis

Immunoadsorption was performed using Globaffin columns (Fresenius Medical Care, Bad Homburg, Germany) on an ADAsorb device (medicap clinic GmbH, Ulrichstein, Germany) together with an AS.TEC 204 centrifuge (Fresenius Medical Care, Bad Homburg, Germany) in nine patients, and with the sheep-anti-human Ig-coated Therasorb columns on the Life 18 device (Miltenyi Biotec, Bergisch Gladbach, Germany) in one patient (patient 9). At least five IA treatments were performed before transplantation until all allogeneic crossmatches, including the CDC B cell and ELISA crossmatches, became negative. In addition, DSA were to be negative in ELISA screening and since March 2009, starting with patient 5, also to be below 1000 MFI in Luminex testing. IA treatments were performed on alternate days and on the last 2 days before transplantation. During each IA treatment, 2.5 plasma volumes per patient were exchanged. Anticoagulation during IA consisted of 1500 units of heparin per hour as a continuous infusion together with sodium citrate (ACD-A, Fresenius Kabi AG, Bad Homburg, Germany) at an infusion rate of 1:23 (citrate infusion:blood flow). Calcium gluconate (10%; B. Braun, Melsungen, Germany) was administered at the venous line to maintain ionized calcium between 0.9 to 1.1 mmol/l. To avoid bleeding complications, the first postoperative IA treatments were accomplished without heparin; therefore, sodium citrate was infused at a rate of 1:16.

Patients 2 and 6 received additional two and five pretransplant plasmapheresis treatments, respectively, to lower unrecognized DSA that might have not responded to IA therapy, such as DSA of the IgM or IgG3 isotype. In patient 2, plasmapheresis was also aimed at lowering anti-Cardiolipin IgM antibodies that did not respond to IA therapy.

After transplantation, IA treatments were continued on alternate days until good allograft function was achieved (e.g. a serum creatinine of less than 2 mg/dl) and DSA were negative in ELISA and below a cut off of 1000 MFI in Luminex testing (since March 2009).

The actual protocol for recipient desensitization is given in Fig. 1. Supplemental Table S1 shows the changes in the protocol over time (2007–2011).

Immunosuppression

Immunosuppression was started before transplantation together with the initiation of IA therapy and consisted of tacrolimus (Astellas, Tokyo, Japan) with a target trough level of 10–12 μg/l, enteric-coated mycophenolic sodium (720 mg twice daily; Novartis, Basel, Switzerland) and methylprednisolone (20 mg/day). At the time of transplantation methylprednisolone was given at a dose of 250 mg, tapered to 20 mg/day by post-transplant day 9.

Immunosuppressive induction therapy was carried out with basiliximab (20 mg on days 0 and 4 after transplantation; Novartis) in six patients and more recently with thymoglobulin (1.5 mg/kg b.w.; Genzyme, Cambridge, MA, USA) in four patients. The latter patients received a

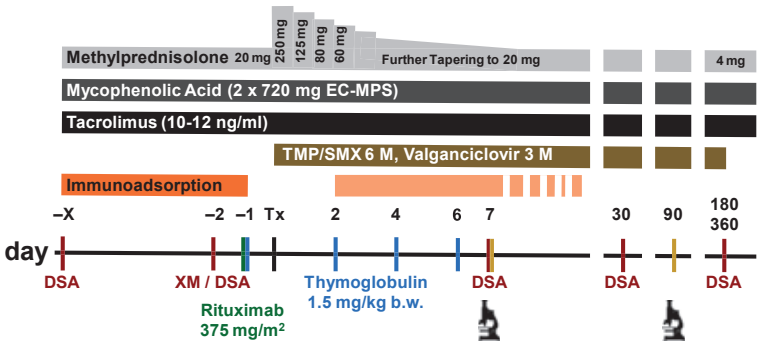

Figure 1 Treatment algorithm for crossmatch-positive living donor kidney transplantation. –X, start of treatment; DSA, donor-specific antibodies; XM, crossmatch; Tx, transplantation; TMP/SMX, trimethoprim and sulfamethoxazole; EC-MPS, enteric-coated mycophenolic sodium.

median of 2.5 (2–4) doses of a median of 113 (75–125) mg of thymoglobulin with a target lymphocyte count of less than 100/nL.

Rituximab (Roche, Basel, Switzerland) was administered at a single dose of 375 mg/m^2, corresponding to a median dose of 645 (100–800) mg, immediately after the last preoperative IA treatment on day –1, when all CDC and ELISA crossmatches had become negative.

Infection prophylaxis

All patients with a transplant from a cytomegalovirus-positive donor received valganciclovir (Roche, Basel, Switzerland) for 3 months. Fungal prophylaxis consisted of 10 ml of nystatin four times daily for 3 months. Pneumocystis jieroveci prophylaxis was conducted by daily administration of trimethoprim (80 mg) and sulfamethoxazole (400 mg) for 6 months as well as a one-time inhalation of pentamidine (300 mg) 24 h after transplantation.

Immunological testing

CDC crossmatches were performed with unseparated peripheral blood mononuclear cells as well as isolated donor T and B lymphocytes using the standard CDC technique without anti-human immunoglobulin enhancement. In addition, the prototype of a solid-phase ELISA crossmatch (AbCross, Biotest, Dreieich, Germany) was used. PRA screenings were performed using CDC and ELISA techniques. DSA of the IgG isotype against HLA antigens were determined by ELISA and, since March 2009 starting with patient 5, also by Luminex technologies using the AbIdent kits of Biotest (Dreieich, Germany), and the LABScreen Single Antigen kit of One Lambda (Canoga Park, CA, USA), respectively. For the detection of DSA of the IgM isotype by Luminex, 1:100 diluted PE-conjugated $F(ab')_2$ fragments of donkey anti-human IgM, Fc antibodies (Dianova, Hamburg, Germany) were used. HLA typings of donors and recipients were performed using PCR-SSP and sequencing. Since May 2009 (patient 7), HLA typing of recipients as well as donors included also HLA-C, -DP and -DQ locus antigens at high resolution.

The HLA alloantibodies were measured after transplantation on days 0, 7, 30, 180, 360 and thereafter every 6 months. Additional testing was performed if deterioration of allograft function was noted.

Diagnosis and treatment of allograft rejection

Until November 2007 (patients 1 and 2), kidney allograft biopsies were only performed in case of suspected allograft pathology (indication biopsies; $n = 8$). Thereafter, protocol biopsies were performed on post-transplant days 7 ($n = 7$) and 90 ($n = 5$) and indication biopsies as required ($n = 10$). In total, in the 10 living donor kidney transplant recipients, 30 biopsies were performed. Biopsy specimens were evaluated according to BANFF 07 criteria [14].

Cell-mediated rejection episodes were treated with 250 mg methylprednisolone for 3 days. In two recipients (patients 4 and 5) who had received basiliximab induction therapy and developed steroid-resistant acute T-cell-mediated rejection, three and four doses, respectively, of thymoglobulin (1.5 mg/kg b.w.) were administered. Patient 6, who experienced severe fever and chills during thymoglobulin induction, received a total of 43 mg of alemtuzumab (MabCampath, Genzyme) for the treatment of a BANFF IA rejection episode.

In three patients with acute antibody-mediated rejection episodes (patients 2, 7 and 8), apheresis treatments were conducted daily until DSA reactivity decreased. In addition, a second dose of rituximab at doses of 700, 650 and 675 mg was administered, respectively.

Two patients in whom either the apheresis therapy for acute antibody-mediated rejection failed (patient 2) or chronic antibody-mediated allograft injury was suspected in the presence of persisting DSA (patient 6) received four infusions of intravenous immunoglobulins (1 g/kg b.w.; KIOVIG, Baxter, Vienna, Austria) every 4 weeks.

Statistical analysis

Data are given as median and range or number and percent. Figures show median and interquartile ranges. Graft survival was calculated according to the Kaplan–Meier method.

Results

Desensitization and post-transplant antibody monitoring

Between March 2007 and September 2010, 10 living donor kidney transplant recipients were desensitized after having given informed consent. Nine of the 10 patients had a positive CDC and/or ELISA crossmatch result prior to IA. The remaining patient (patient 8; Table 2) had strong DSA as detected by Luminex and ELISA testing, however, crossmatch results were negative before desensitization. One patient had DSA against a repeat mismatch to a previous transplant (patient 5; HLA-B44), whereas in three patients alloantibodies were induced subsequent to blood transfusion therapy or pregnancy. In three out of seven retransplant recipients, repeat mismatches could not be excluded attributable incomplete typing of the donor from the previous transplant. Baseline characteristics of the 10 patients are summarized in Table 1. Results of antibody screenings and crossmatch tests are depicted in Table 2.

Table 1. Baseline characteristics.

Recipient characteristics	
Female gender – *n* (%)	3 (30)
Age (years) – median (range)	44 (32–53)
Caucasian race – *n* (%)	10 (100)
Cause of end-stage renal disease – *n* (%)	
Diabetes or hypertension	0 (0)
Glomerulonephritis	4 (40)
Other/unknown	6 (60)
Mode of pretransplant dialysis – *n* (%)	
Hemodialysis	9 (90)
Peritoneal dialysis	1 (10)
Time on dialysis (years) – median (range)	4 (1–13)
Number of previous kidney transplants (*n*) – 0/1/2	3/6/1
Waiting time for last kidney transplant (years) – median (range)	3 (1–10)
Donor characteristics	
Related donor – *n* (%)	1 (10)
Female gender – *n* (%)	6 (60)
Donor age (years) – median (range)	48 (25–68)
HLA-A+B+DR mismatches* – median (range)	4 (0–6)
Perioperative procedure and follow-up	
Preoperative apheresis (*n*) – median (range)	10 (5–23)
Preoperative rituximab dose (mg) – median (range)	645 (100–800)
CD19 + cells on day 30 (/μL) – median (range)	5 (0–39)
Patients with basiliximab induction – *n* (%)	6 (60)
Patients with thymoglobulin induction – *n* (%)	4 (40)
Postoperative apheresis (*n*) – median (range)	7 (2–18)
Postoperative hospital stay (days) – median (range)	23 (13–57)
Clinical follow-up (months) – median (range)	19 (3–44)

*According to Eurotransplant criteria.

Before desensitization, a median of two (0–6) different Luminex-detected DSA against HLA-A, -B, -C, -DR, -DQ or -DP antigens of the donor with a reactivity of greater than 1000 MFI were detectable in the patients' serum (Table 2), with a median MFI (highest per serum) of 6203 (0–19 008) (Fig. 2a). One patient (patient 4) had no detectable HLA alloantibodies, however, was believed to have alloantibodies against non-HLA antigen systems referable to positive B-cell crossmatch results in the absence of auto-antibodies. After a median of 10 (5–23) IA treatments, all patients were successfully desensitized with negative CDC B cell and ELISA crossmatch results and without detectable DSA in ELISA testing. The median MFI of Luminex-DSA was 891 (0–6588) (Fig. 2a). Patients received a median of 7 (2–18) post-transplant IA treatments. Median MFI of Luminex-detected DSA at last follow-up was 149 (0–19 799). Figure 2b shows that after a median of 10 pretransplant IA treatments there was a decrease of immunoglobulins, with a 98%, 57%, and 77% reduction of IgG, IgA and IgM, respectively. Figures 2c and d show in individual patients the development of the maximum Luminex-detected HLA class I and class II DSA, respectively. Eight of the 10 patients had a maximum Luminex-detected DSA of 1000 MFI or below at the time of transplantation. Only patient 2, who was transplanted before the introduction of routine Luminex testing, and patient 6, who was transplanted before the start of routine donor typing for HLA-C, -DP, and -DQ locus antigens, were found retrospectively to have Luminex-DSA >1000 MFI at the time of grafting.

Figure 2e depicts the evolution of Luminex-detected DSA before and after transplantation in patient 5. After a total of 22 IA treatments, all crossmatches became negative in this patient and he was transplanted. DSA against HLA-B35 and -B44 were undetectable in the patient's serum immediately before transplantation. In contrast, HLA-Cw7, which was recognized retrospectively as DSA after complete DNA typing of the donor and therefore was not considered during recipient desensitization, was not eliminated from the patient's circulation. Despite relatively high DSA reactivity against HLA-Cw7, which we believe was a reaction against the denatured Cw7 antigen on the bead surface, with an MFI of 10 734 at the time of transplantation, all crossmatches, including the ELISA crossmatches, turned negative.

Figure 2f shows anti-Cardiolipin IgG and IgM antibodies in relation to apheresis therapy in patient 2 who was originally diagnosed with systemic lupus erythematosus and catastrophic antiphospholipid syndrome. Anti-Cardiolipin antibodies of the IgG isotype responded well to IA therapy (85% reduction during pretransplant desensitization), whereas anti-Cardiolipin antibodies of the IgM isotype were reduced only by about 40% despite intensified treatment by IA and plasmapheresis.

Graft survival and function

The 2-year graft survival rate of the 10 living donor kidney transplants was 100% (Table 3). Patient 2, who will be reported in detail below, lost her allograft 25 months after transplantation and the patient returned to hemodialysis. All other patients in this series were alive with a functioning allograft at last visit.

Figure 3 shows serum creatinine (a), MDRD-GFR (b) and protein to creatinine ratio (c) in the 10 living donor kidney transplant recipients up to post-transplant day 540. Median serum creatinine at last visit was 1.6 (0.9–2.8) mg/dl, median MDRD-GFR 54 (21–70) ml/min/1.73 m^2, and median urinary protein to creatinine ratio 0.1 (0–0.8).

Biopsy-proven acute rejection

Borderline changes were often found in day 7 ($n = 4$) and day 90 ($n = 2$) protocol biopsies. BANFF IA acute T-cell-mediated rejection was observed in two patients

Table 2. Antibody screening and crossmatch results.

	Tx		CDC crossmatch				ELISA crossmatch		Pre Tx DSA (ELISA)	Pre Tx DSA (Luminex)	Apheresis		Post Tx DSA (Luminex)	AMR	Last SCr
Pt	Date	*n*	T cell [score*]	B cell [score*]	U cell –DTT [score*]	U cell +DTT [score*]	Class I [O.D†]	Class II [O.D.†]	[allele]	[allele (MFI)]	Pre Tx [N]	Post Tx [N]	*De novo*/persistent [class]	Yes/No	[mg/dl]
1	03/07	2	Positive 4	Positive 8	Positive 4	Positive 4	Positive 664		Positive B7, B60	Positive B7 (10 111), B60 (7282)	8	8	–	No	2.5
2	11/07	2		Positive 6	Positive 4					Positive DR1 (4013), DQB1*05 (3048), DPB1*02:01 (9486)	10	18	Persistent class II	Yes	HD (after 2 years)
3	09/08	2		Positive 4						Positive A3 (1432), A29 (2919)	5	6	–	No	1.2
4	11/08	3		Positive 4							5	11	–	No	1.4
5	03/09	2	Positive 6	Positive 6	Positive 4	Positive 8	Positive 388		Positive B44	Positive B35 (19 008), B44 (17 682)‡, Cw7 (19 906)§	22	3	–	No	1.6
6	05/09	2		Positive 4				Positive 86	Positive DQB1*07	Positive DQB1*03:01 (14 509), DQA1*05:05 (13 581)	23	10	Persistent class II	No**	2.8
7	05/09	1	Positive 8	Positive 8	Positive 8	Positive 8	Positive 375	Positive 132	Positive A1	Positive A1 (2335)	12	7	Persistent class I *De novo* class II	Yes††	0.9
8	11/09	2							Positive B18, B37	Positive B18 (9676), B37 (2516), Cw7 (3279), DRB1*16:01 (4798), DR51 (1653), DQB1*06:02 (1156)	8	2	Persistent class I and class II	Yes	1.9

Table 2. continued

	Tx		CDC crossmatch				ELISA crossmatch		Pre Tx DSA (ELISA)	Pre Tx DSA (Luminex)	Apheresis		Post Tx DSA (Luminex)	AMR	Last SCr
Pt	Date	*n*	T cell [score*]	B cell [score*]	U cell –DTT [score*]	U cell +DTT [score*]	Class I [O.D†]	Class II [O.D.†]	[allele]	[allele (MFI)]	Pre Tx [N]	Post Tx [N]	*De novo*/persistent [class]	Yes/No	[mg/dl]
9	03/10	1	Positive 4	Positive 4	Positive 4				Positive Cw*12:03*	Positive Cw*12:03 (1760)¶	10	6	–	No	1.7
10	09/10	1	Positive 4							Positive DRB1*13:01 (1164) ¶	9	5	–	No	1.3

*Scores reflect the strength of donor-specific alloantibodies: 4: 21–50%; 6: 51–80%; and 8: 81–100% lysed cells.
†O.D., optical density reflects the strength of donor-specific alloantibodies.
‡Alloantibodies against repeat HLA mismatches.
§Most likely an antibody against a denatured antigen.
¶IgM antibody.
**Suspected chronic antibody-mediated rejection.
††Diagnosed in 3 months protocol biopsy.
Pt, patient; Tx, transplantation; CDC, complement-dependent cytotoxicity; DSA, donor-specific antibody; AMR, antibody-mediated rejection; U, unseparated cells; DTT, dithiothreitol; MFI, mean fluorescence intensity; SCr, serum creatinine; HD, hemodialysis.

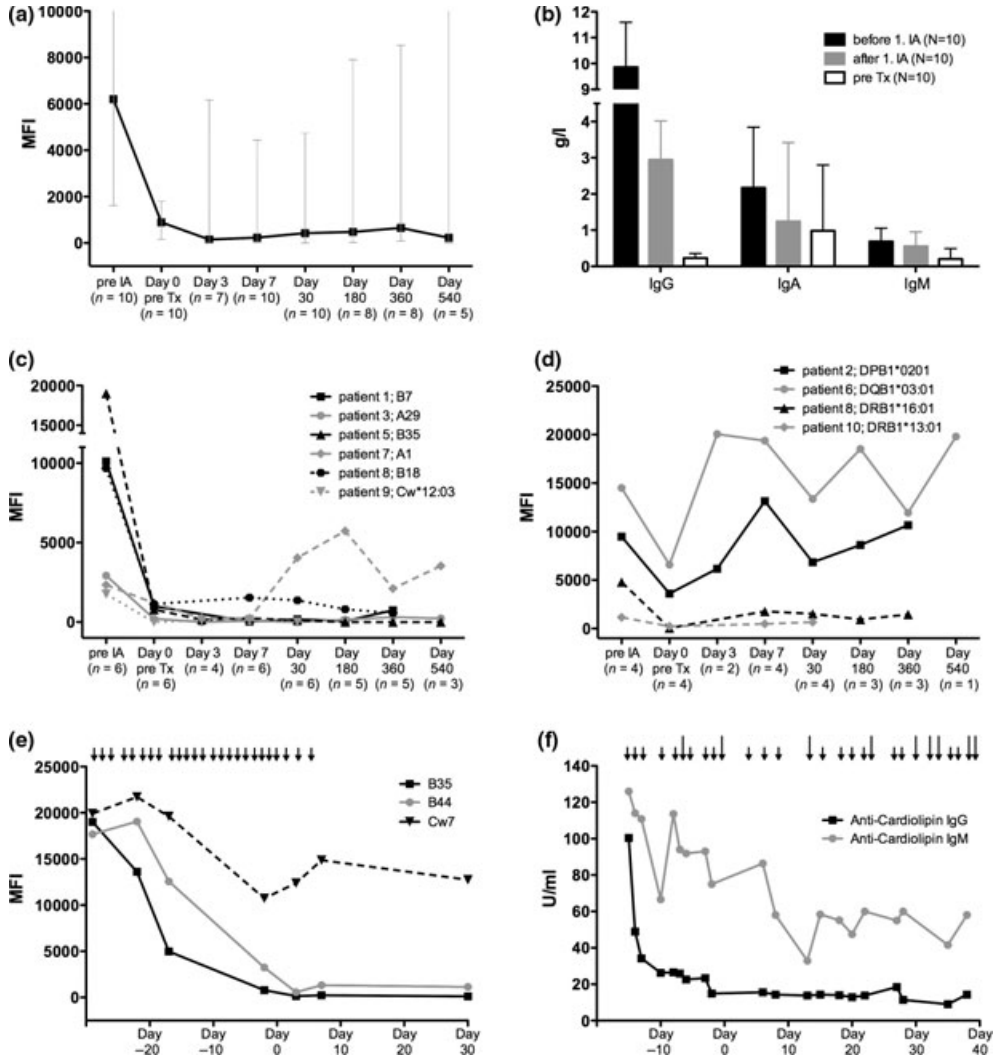

Figure 2 (a) Luminex-detected donor-specific antibodies (DSA) before first pretransplant IA (pre IA), before transplantation (day 0, pre Tx) and to day 540 after transplantation. Highest mean fluorescence intensity (MFI) value per serum is recorded. (b) Decrease of immunoglobulins during the first IA treatment and during a median of 10 pretransplant (pre Tx) IAs. Median values and interquartile ranges are shown. (c) and (d) Development of individual Luminex-detected DSA before the first pretransplant IA (pre IA), before transplantation (day 0, pre Tx) and to day 540 after transplantation. Highest MFI value per serum is recorded for HLA class I (c) and HLA class II DSA (d) separately. (e) Evolution of Luminex-detected DSA before and after transplantation in patient 5 in relation to IA (↓) treatment. Pre IA DSA reactivities are shown. The reactivity against HLA-Cw7 was most likely ascribable to a reaction against denatured HLA that was recognized as donor-specific only retrospectively and therefore not considered during desensitization. (f) Anti-Cardiolipin IgG and IgM antibodies in patient 2 in relation to IA (short ↓) or plasmapheresis (long ↓) treatment. Pre apheresis antibody results are shown. Efficient removal of anti-Cardiolipin IgG (normal value <10 U/ml) but not anti-Cardiolipin IgM (normal value <5 U/ml) antibodies by the apheresis procedure.

(patients 1 and 6) in indication biopsies. A total of five biopsies in three patients (patients 2, 7 and 8) showed signs indicative for acute antibody-mediated rejection. This included ATN-like minimal inflammation in three biopsies and glomerular inflammation in two biopsies (Table 3).

Patient 2, who had systemic lupus erythematosus and catastrophic antiphospholipid syndrome, showed lesions indicative of hemolytic-uremic syndrome in a biopsy specimen obtained on day 157 after transplantation. At that time antiphospholipid antibodies were at low levels (anti-Cardiolipin IgG: 17 U/ml; anti-Cardiolipin IgM: 3 U/ml) and the patient had stable tacrolimus trough levels at about 7 μg/l. In contrast, preexisting DSA against DQB1*05 (1033 MFI) and DPB1*02:01 (8625 MFI) were detectable and the patient was found to have glomerulitis

Table 3. Outcome and complications.

Outcome		
2-Year graft survival – %	100	
Acute allograft rejection		
Patients with at least one acute rejection episode – *n* (%)		
Antibody-mediated changes (C4d + DSA)	3 (30)	
Borderline changes	9 (90)	
Acute T-cell-mediated rejection (BANFF IA)	2 (20)	
Individual biopsy results – *n*	*Ind*	*Prot*
Acute antibody-mediated changes (C4d + DSA)		
No signs of rejection	0	0
I.	2	1
II.	2	0
III.	0	0
Borderline changes	8	6
Acute T-cell-mediated rejection	2	0
Complications		
Delayed graft function* – *n* (%)	1 (10)	
Patients with infectious complications – *n* (%)		
Viral		
Polyomavirus BK		
Nephropathy (SV40-positive)	0 (0)	
Replication (>10^4 copies/ml plasma)†	2 (20)	
Cytomegalovirus	1 (10)	
Bacterial		
Urinary tract infection	2 (20)	
Pneumonia	1 (10)	
Wound infection	1 (10)	
Central venous catheter infection	2 (20)	
Fungal		
Pneumonia	0 (0)	

*As defined by dialysis within the first post-transplant week.
†Necessitating reduction in immunosuppression.
DSA, donor-specific antibody; Ind, indication biopsy; Prot, protocol biopsy.

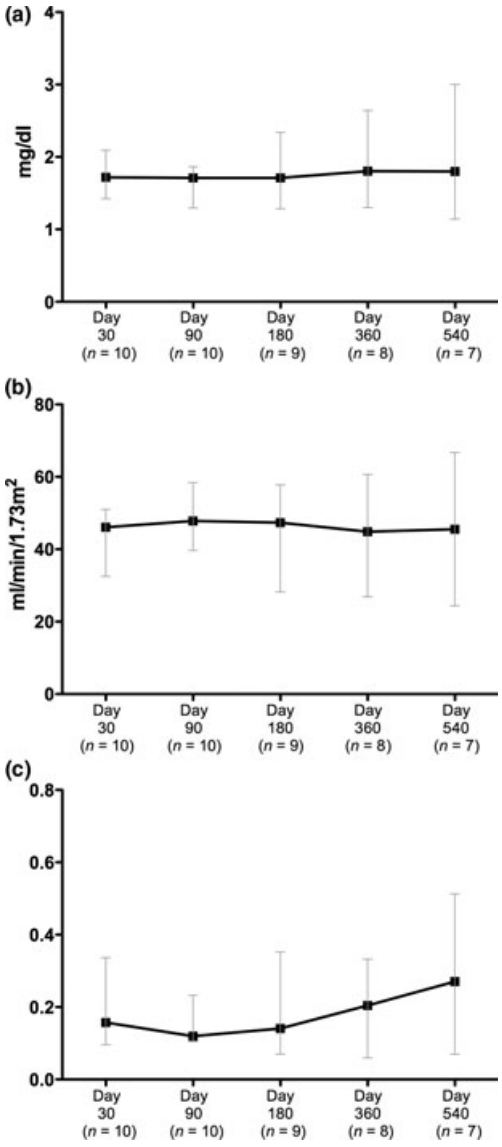

Figure 3 (a) Serum creatinine, (b) MDRD-GFR and (c) protein to creatinine ratio in 10 living donor kidney transplant recipients with a positive crossmatch or strong DSA. Median values and interquartile ranges up to day 540 post-transplant are shown. To convert values for serum creatinine to micromoles per liter, multiply by 88.4. MDRD-GFR, glomerular filtration rate as estimated by the MDRD formula.

and C4d-positivity in the same biopsy specimen so that the diagnosis of antibody-mediated rejection was established. After a total of nine plasmapheresis treatments followed by four monthly infusions of intravenous immunoglobulins (1 g/kg b.w.), kidney function stabilized but remained impaired with a serum creatinine of 4.8 mg/dl at year 1 and the graft was eventually lost beyond year 2 after transplantation.

Patient 7 showed evidence of antibody-mediated rejection only in the 3-months protocol biopsy when the serum creatinine was 1.2 mg/dl. At this time, the patient developed, in addition to preexisting class I DSA against HLA-A1 with an MFI of 5736, *de novo* DSA against class II. After treatment with rituximab and an additional 7 IA treatments, DSA against HLA-A1 was reduced to 2109, and *de novo* class II DSA were reduced from 6248 to 2027 MFI for the specificity HLA-DR1, from 1412 to 0 MFI for HLA-DQB1*06, and from 1412 to 0 MFI for HLA-DQA1*01. Serum creatinine at last visit in this patient was 0.9 mg/dl 18 months after transplantation.

Patient 8 left the hospital against medical advice on postoperative day 13 after only two post-transplant IA treatments and with a serum creatinine of 1.6 mg/dl. He was readmitted on day 43 post-transplant with a serum creatinine of 5.3 mg/dl. The biopsy revealed acute antibody-mediated rejection. Luminex-detected preexisting

DSA against HLA-Cw7 had increased from 2603 MFI at day 7 to 10 559 MFI. After a total of 15 IA treatments and a second dose of rituximab, DSA against HLA-Cw7 decreased to 3667 MFI (day 180) and kidney function recovered with a current serum creatinine 11 months after transplantation of 1.9 mg/dl.

Patient 6 had an increase of DSA immediately post-transplant that persisted and on last assessment on day 540 the DSA were directed against the donor HLA antigens DQB1*03:01 and DQA1*05:05 at 19 799 and 19 799 MFI, respectively. Although classical features of antibody-mediated rejection could not be detected in serial allograft biopsies (negative C4d), there was significant interstitial fibrosis and tubular atrophy. In the presence of impaired kidney function, chronic antibody-mediated rejection was suspected and the patient received four intravenous infusions of immunoglobulins (1 g/kg b.w. every 4 weeks). At last visit, serum creatinine had stabilized at 2.8 mg/dl.

Infection and adverse events

Table 3 summarizes infections and adverse events in the 10 transplant patients. Delayed graft function, defined as need for dialysis within the first week post-transplant, was observed in 1 of 10 patients (patient 1). Infectious complications including cytomegalovirus reactivation ($n = 1$), urinary tract infection ($n = 2$), pneumonia ($n = 1$), wound infection ($n = 1$) or central venous catheter infection ($n = 2$) were infrequent. Polyomavirus JC infection was not detected in any of the patients.

Two patients (patients 1 and 5) had polyomavirus BK replication with more than 10^4 copies/ml plasma. Reduction of immunosuppression was sufficient in both patients to clear the virus. In allograft biopsies there was no evidence for BK virus nephropathy, as indicated by negative SV40-staining.

Estimated cost for IA with reusable Globaffin columns

An estimate for treatment costs with the Globaffin columns is given in the supplemental Table S2. With 17 IA treatments (median number in this study), the cost per single IA including disposables and tax is 1328 €. The cost decreases to 903 € per treatment when 33 IA treatments are performed (maximum number in this study).

Discussion

Recipient desensitization

Crossmatch-positive living donor kidney transplantation can be accomplished using different desensitization strategies such as plasmapheresis or intravenous immunoglobulins [1,2]. However, despite these efforts, crossmatch-positive living donor kidney transplantation is associated with inferior graft survival as to transplantation in non-sensitized patients [3–7]. Our group demonstrated that repeated IA is capable of eliminating even strong DSA and to render a positive CDC crossmatch negative [8]. We now present data on a cohort of 10 desensitized living donor kidney recipients with a median of 19 months of follow-up. Graft and patient survival rates 1 and 2 years after transplantation were 100% and considerably higher than in comparable high risk patients in whom 1-year patient survival for CDC crossmatch-positive patients was reported to be 88% [15]. Although our results were obtained in a rather small cohort of patients in an uncontrolled study with short- to medium-term follow-up, to our knowledge, this is the first report which describes in detail the efficacy of repeated pretransplant IA treatment for desensitization of crossmatch-positive living donor kidney transplant recipients.

We chose IA for recipient desensitization since IA, in contrast to plasmapheresis, allows the exchange of large plasma volumes. In their study of IA in deceased donor kidney recipients, the Vienna group showed that up to 11 liters of plasma may be processed during preoperative IA treatment. An initially positive CDC crossmatch could be rendered negative in 21 patients [16]. Patients in our study received a median of 10 IA treatments pretransplant, resulting in an approximately 90% reduction of DSA (Fig. 2). It is unlikely that these rates would have been achieved with plasmapheresis and administration of intravenous immunoglobulins alone as indicated by a recent study on 13 living donor kidney transplant recipients of whom three patients were not successfully desensitized [17]. In our study, all 10 patients were successfully desensitized and transplanted after IA.

In early studies on the use of IA for recipient desensitization, no post-transplant IA was performed to combat the deleterious influence of rapid antibody rebound. Graft survival in these studies was as low as 54% after a median of 26 months after transplantation [18]. It must be borne in mind that following IA usually a rebound of antibodies is seen. Therefore, we performed repeated post-transplant IA to avoid antibody rebound. In addition, the patients received powerful immunosuppression, plus a one-time administration of rituximab immediately pretransplant. Whereas anti-CD20 therapy has no immediate effect on antibody-producing plasma cells, it may prevent *de novo* synthesis of alloantibodies by means of blockade of antigen presentation [19].

It was to some extent surprising that, with the combination of transient post-transplant IA and powerful immunosuppression, DSA remained below the threshold of 1000 MFI in Luminex testing in six of the recipients during the complete follow-up period. *In vivo* adsorption

of low-level DSA by the allograft could be an additional mechanism that might explain the persistent low alloantibody levels after crossmatch-positive transplantation.

Precise knowledge of alloantibodies in the pre- and post-transplant phase

Precise knowledge of the recipient's alloantibody status before transplantation and antibody monitoring post-transplant is a prerequisite for the adequate management of these patients who are at high risk of antibody-mediated rejection. Consequently, we have expanded the HLA characterization of recipients and donors since May 2009 and are currently typing also for HLA-C, -DP and -DQ locus antigens at high resolution [20,21]. In two patients with positive crossmatch results (patients 6 and 9), such HLA antibodies were the only detectable HLA alloantibodies.

Recent reports suggest that preexisting donor-specific HLA antibodies detected exclusively by the highly sensitive Luminex technology are not necessarily associated with graft rejection [22–25]. The validity of this suggestion is impressively shown for patient 5 (Fig. 2e). In this patient, DSA of specificity HLA-Cw7 was detected only retrospectively, after complete donor and recipient high resolution DNA typing had been performed. Had we based our decision on these Luminex results, the patient would not have been transplanted. However, at the time of transplantation the crossmatches were negative and the known antibodies had been eliminated. Despite persistence of HLA-Cw7 after transplantation at high levels (9963 MFI on day 540), this patient never suffered from antibody-mediated rejection and had good allograft function at last visit. One possible explanation is that an antibody reaction against a denatured HLA-Cw7 antigen on the bead surface was detected by Luminex that has no clinical relevance [26]. Nevertheless, we decided to incorporate Luminex testing into our treatment algorithm in March 2009 (starting with patient 5) to minimize the risk of antibody-mediated allograft injury in this cohort of living donor kidney recipients. We chose a cutoff of 1000 MFI because that had been used by others [27,28].

T-cell and antibody-mediated rejection

Although severe T-cell-mediated rejection was infrequent, borderline changes occurred in nine out of 10 patients. In addition, two of six patients with basiliximab induction required treatment with lymphocyte depleting antibodies during follow-up. As a consequence, induction therapy with thymoglobulin was introduced in May 2009.

Antibody-mediated rejection was observed in three of 10 patients. Patients had either persistently elevated DSA at the time of diagnosis of antibody-mediated rejection episode (patients 2 and 8) or, in addition to persistent DSA, *de novo* HLA class II DSA (patient 7) (Table 2). However, in one of the recipients antibody-mediated rejection was in all likelihood the result of patient incompliance and insufficient immunosuppression (patient 8), which has only recently been identified as a major cause of antibody-mediated allograft injury and allograft loss [29,30]. In another patient features of antibody-mediated rejection were only found in the 3-month protocol biopsy (patient 7). In two patients with evidence of antibody-mediated rejection (patients 7 and 8), DSA could be eliminated after additional treatment with IA and rituximab, and DSA did not reappear during subsequent follow-up. In contrast, repeated IA and plasmapheresis, as well as administration of intravenous immunoglobulins had only transient effects on antibody levels in patient 2, and the patient eventually lost her allograft beyond year 2 after transplantation.

One patient (patient 6) with persistently high DSA and significant interstitial fibrosis and tubular atrophy but negative C4d in repeated allograft biopsies had impaired allograft function and was treated with four monthly infusions of intravenous immunoglobulins even though he was not fulfilling the classical criteria for antibody-mediated allograft injury. However, only recently has it been recognized that antibody-mediated rejection often is C4d negative and that antibody-mediated allograft injury may be missed by current diagnostic tools [31,32].

Notably, all crossmatch-positive patients ($n = 4$) who had persistent or *de novo* Luminex-detected DSA after transplantation had suspected or biopsy-proven antibody-mediated rejection, except for patient 5 who was believed to have an antibody against denatured HLA-Cw7. Therefore, at least in this small patient cohort of desensitized crossmatch-positive patients, all DSA that were detected after transplantation, irrespective whether the antibodies were preexisting or *de novo*, appeared to have clinical relevance. This is at variance with recent work in which only *de novo* alloantibodies (mostly HLA class II) were claimed to have a negative effect on graft survival. However, these data were not from a crossmatch-positive patient cohort and the antibody-mediated rejection episodes occurred 7 days to 31 years post-transplant [33]. The findings from our study, together with recent studies from other groups, highlight the importance of HLA alloantibody monitoring after crossmatch-positive kidney transplantation [4].

Infectious complications

Although our patients were heavily immunosuppressed and in addition received IA with an associated strong reduction of immunoglobulin levels, infectious complications

did not appear to be increased (Table 3). This is in line with recent findings showing that repeated IA without intravenous substitution of immunoglobulins is feasible without the risk of excessive infectious complications [16,34]. As a note of caution, we recently changed the protocol and now administer thymoglobulin instead of basiliximab as induction therapy. Little is known about the effects of combined administration of two depleting antibodies (thymoglobulin and rituximab) with regard to occurrence of infections, such as cytomegalovirus and BK virus. So far, however, we did not notice an increase of infection rates in a small patient series (Table 3).

Conclusions

We describe a treatment algorithm for the desensitization of living-donor kidney transplant recipients that allows the rapid (and durable) elimination of DSA by repeated pre- and post-transplant IA. In our experience the combination of peritransplant apheresis with potent immunosuppression and anti-CD20 therapy prevented the re-emergence of antibodies as well as *de novo* antibody production in the majority of patients. The rate of side effects was low. Short- to medium-term graft outcome was good; however, final assessment of this treatment strategy requires experience with larger patient cohorts with longer post-transplant follow-up.

Authorship

CM and CS: research/study design, writing of the article, performance of the research/study, data analysis. GO and MZ: research/study design, writing of the article, data analysis. SS, SMG and KK: performance of the research/study. JB, JS, CS, VS: research/study design, performance of the research/study.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Protocol changes over time (2007–2011).

Table S2. Estimated costs for immunoadsorption (catalog price).

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