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Postoperative rebound of antiblood type antibodies and antibody-mediated rejection after ABO-incompatible living-related kidney transplantation

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Summary

The purpose of this study is to examine whether postoperative antiblood type antibody rebound is attributed to kidney allograft rejection in ABO blood type-incompatible (ABO-I) living-related kidney transplantation (KTx). A total of 191 ABO-I recipients who received ABO-I living-related KTx between 2001 and 2013 were divided into two groups: Group 1 consisted of low rebound [(≦1:32), $N = 170$] and Group 2 consisted of high rebound [(≧1:64), $N = 21$], according to the levels of the rebounded antiblood type antibodies within 1 year after transplantation. No prophylactic treatment for rejection was administered for elevated antiblood type antibodies, regardless of the levels of the rebounded antibodies. Within 1 year after transplantation, T-cell-mediated rejection was observed in 13 of 170 recipients (13/170, 8%) in Group 1 and in 2 of 21 recipients (2/21, 10%) in Group 2 (Groups 1 vs. 2, $P = 0.432$). Antibody-mediated rejection was observed in 15 of 170 recipients (15/170, 9%) and 2 of 21 recipients (2/21, 10%) in Groups 1 and 2, respectively ($P = 0.898$). In this study, we found no correlation between the postoperative antiblood type antibody rebound and the incidence of acute rejection. We concluded that no treatment is necessary for rebounded antiblood type antibodies.

Introduction

A shortage of organ donors for transplantation has become a serious problem throughout the world. To overcome this problem, transplantation across the ABO blood type barrier has been widely performed [1–7]. Transplantation across the blood type barrier provided the first definitive evidence that hyperacute rejection is caused by natural occurring antibodies, so-called antiblood type antibodies. As described more than 10 years ago [8], we reported four patterns of changes in antiblood type antibodies in ABO-incompatible (ABO-I) kidney recipients treated with splenectomy and conventional triplicate immunosuppressive regimens such as cyclosporine and azathioprine. At that time, we found a clear association between post-transplant anti-ABO blood type antibody titres (anti-ABO titres) and graft survival [9]. The development of new immunosuppressive regimens, however, has yielded excellent results in ABO-I with no association between anti-ABO titres and graft survival rates [10]. With the advent of novel desensitization protocols, including the combination of tacrolimus (TACROLIMUS)/mycophenolate mofetil (MMF) and/or anti-CD 20 antibody rituximab, the graft survival in ABO-I increased over 90% at 10 years after transplantation, which

is comparable to that in ABO blood type-compatible cases (ABO-C) [11]. However, the role of postoperative anti-ABO titres in acute rejection under our current immunosuppressive regimen remains unclear. Some institutions recommend postoperative therapeutic plasma exchange to prevent antibody-mediated rejection (AMR) [12]. However, accommodation or even tolerance to incompatible blood type has been observed in ABO-I transplantation and the necessity of prophylactic treatment for postoperative elevation of antiblood type antibodies has been controversial in ABO-I KTx [13–15]. We therefore investigated the relevance of the postoperative anti-ABO titre rebound and acute rejection in ABO-I KTx to conclude the necessity of B cell-targeting therapies for the rebounded anti-ABO antibodies.

Materials and methods

Written informed consent was obtained from all patients in the renal transplant programme who were enrolled in this study. Clinical and laboratory data were extracted from our electronic database and the patient's medical records.

Patients

Between 2001 and 2013, we performed 206 ABO-I at the Department of Urology, Tokyo Women's Medical University. In total, 11 recipients died of cerebrovascular infarction ($N = 5$), cardiac failure ($N = 5$) or sudden death ($N = 1$) (Tables 1 and 2). Data were not obtained in four patients who were followed up in other hospitals. The data of the remaining 191 recipients were studied. Antiblood type antibodies (IgM/IgG) were regularly monitored until 1 year after transplantation. We divided the patients whose ABO titres rebounded within 1 year after transplantation into two groups: Group 1, low rebound (≦1:32) after transplantation, $N = 170$; Group 2, high rebound (≧1:64), $N = 21$. Table 1 shows the patient characteristics of both groups. No significant differences in age, gender ratio, haemodialysis duration, sensitized status, graft loss, death and immunosuppressive regimens including B cell-targeting therapies (splenectomy or/and rituximab administration), follow-up periods or ABO-incompatibilities were observed between the two groups. Antiblood type titres at baseline was significantly higher in Group 2 ($P = 0.011$). As shown in Table 2, there was also no significant difference in patients' original disease between the two groups.

Table 1. Patient characteristics, according to rebound titers after Tx.

	Total	Group 1; ≦32	Group 2; ≧64	*P* value
N	191	170	21	–
Gender (M/F)	118/73	105/65	13/8	0.990
Age (years)	46.2±13.5	47.0±13.5	40.0±12.0	0.125
HD duration (months)	47.3±53.3	46.9 ±52.6	50.4±60.1	0.781
Primary Tx/ More than twice Tx	179/12	159/11	20/1	0.749
Number of HLA mismatches (HLA-AB, DR)	2.1 ±1.1 1.0±0.7	2.1±0.8 1.0±0.6	1.7± 1.0 0.9±0.4	0.268 0.445
DSA Luminex single (%)	17.1 ±24.3	16.8±22.5	18.6±14.8	0.433
Anti-blood type titers at baseline	56.2±109	51.2±18.7	112.1 ± 12.3	0.011
Graft loss (*n*, %)	14, 7.3%	11, 6.5%	3, 14.0%	0.104
Death (*n*, %)	1, 0.5%	1, 0.6%	0, 0 %	0.725
Immunosuppression				
Spx	42 (22.0%)	35 (20.5%)	7 (33.3%)	0.187
Rit	140 (73.2%)	127 (74.7%)	13 (62.0%)	0.108
Spx & Rit	9 (5%)	8 (5%)	1 (5%)	–
CD25Ab	162 (84.8%)	146 (85.9%)	16(76.2%)	0.243
Follow up (months)	67±43	67±43	74±46	0.490
Blood incompatibilities				
A→B	32 (18%)	30 (18%)	2 (10%)	–
A→O	52 (28%)	41 (24%)	11 (50%)	–
B→A	20 (10%)	20 (12%)	0 (0%)	–
B→O	39 (20%)	37 (22%)	2 (10%)	–
AB→A	25 (13%)	24 (14%)	1 (5%)	–

Table 2. Patient original disease, according to rebound titers after Tx.

	Total	Group 1: ≦32	Group 2: ≧64	*P* value
N	191	170	21	–
CGN	45 (23.6%)	40 (23.5%)	5 (23.8%)	0.811
DM nephropathy	26 (13.6%)	22 (12.9%)	4 (19.0%)	0.566
Polycystic disease	10 (5.2%)	10 (5.9%)	0 (0%)	NA
FSGS	8 (4.2%)	7 (4.1%)	1 (4.8%)	NA
Nephrosclerosis	2 (1.0%)	2 (1.2%)	0 (0%)	NA
IgA nephropathy	42 (22%)	37 (21.8%)	5 (23.8%)	0.654
Lupus nephropathy	2 (1.0%)	1 (0.6%)	1 (4.8%)	NA
Unknown	40 (20.9%)	37 (21.8%)	3 (14.3%)	0.502

Current immunosuppressive medications for ABO-I

Tacrolimus (Prograf®, Astellas Fujisawa, Osaka, Japan) was administered 7 days before transplantation, at 0.15 mg/kg/day and adjusted to maintain a tacrolimus trough level in whole blood of between 8 and 10 ng/ml for 1 or 2 months postoperatively, between 7 and 9 ng/ml until 1 year and between 4 and 6 ng/ml thereafter (Fig. 1). Mycophenolate mofetil (MMF, Cellcept®, Roche, Nutley, NJ, USA) was also administered 7 days before transplantation at a dose of 2000 mg/day and was tapered to 1000–1500 mg/day by 1 month postoperatively. Methylprednisolone (MP, Medorol®, Pfizer, Tokyo, Japan) was also started 7 days

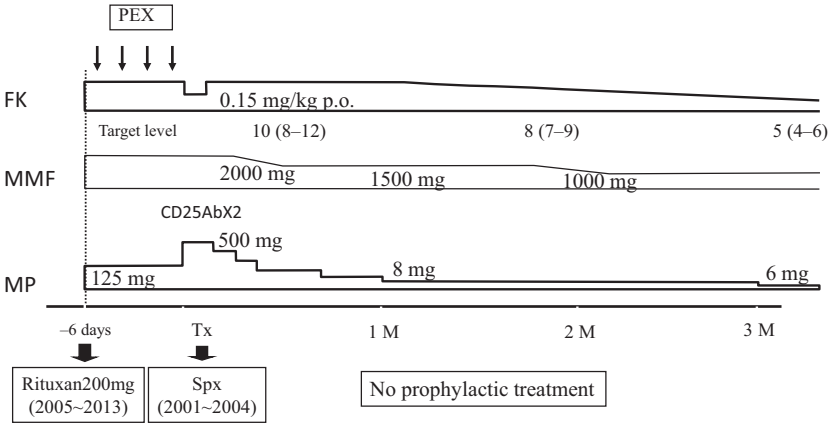

Figure 1 Immunosuppressive regimen for recipients with ABO incompatibilities at TWMU (2001–2013).

before transplantation at a dose of 125 mg/day. On the day of operation, the dose of MP was increased to 500 mg/day and then tapered to 6–8 mg/day within 1–2 months after transplantation and to 4 mg/day thereafter. We do not adopt steroid-free protocol in any recipients. Between 2001 and December 2004, splenectomy was performed, which was replaced with a single dose 200 mg/day rituximab (Rit, Rituxan®, Zenyaku Kogyo, Niigata, Japan) on day 7 after 2005. Some patients received both splenectomy and rituximab. Intravenous immunoglobulin was not administered to any recipient in this study.

Removal of serum anti-A and/or anti-B blood type antibodies and monitoring antibodies

To remove antiblood type antibodies, recipients received three or four sessions of double plasmapheresis (DFPP) before transplantation, according to our previous protocol [11]. Briefly, DFPP was performed using OP-05H (ASAHI Medical Co. Ltd., Osaka, Japan) and Evaflux 2A (Kuraray Co. Ltd., Osaka, Japan) plasma separators until blood type antibodies titres decreased to a level of 1:32 or below. The number of DFPPs prior to transplantation was determined by the baseline titres of antiblood type antibodies. Currently, we consider titres of up to 1:32 as being within acceptable range: when the titre was 1:256 or more, 4 sessions of DFPP were indicated; when the titre was less than 1:128, 3 sessions of DFPP were performed. Antiblood type IgG antibodies are difficult to detect by the hemagglutination assay, because the major isotype-facilitating red cell agglutination is pentameric IgM. We have developed an ELISA assay for the identification of antiblood type IgG antibodies. In this study, levels of IgM anti-A and anti-B antibodies were determined using the saline and/or Bromerin agglutination techniques, as specified in the protocol. The indirect Coomb's test was used to measure IgG titres as the ELISA assay is not yet accepted worldwide. Antiblood type antibodies were regularly measured every other month.

Detection of antidonor HLA antibody using a single phase assay (SPA, Luminex)

Before transplantation, we determined the sensitized status of all patients using a lymphocyte cytotoxic test (LCT)/flowcytometric cross-match test (FCXM)-cross-match assays, as previously reported [11]. Patients with positive LCT/FCXM-cross-match assay results were excluded from this study. SPA has been used at our centre since 2005; thus, the DSA status of the recipients who underwent transplantation prior to 2005 was analysed retrospectively using serum that had been stored at −80°C, as previously reported. Briefly, 20 μl of sera was added to 5 μl of class I or class II antigen beads. The beads were then incubated in the dark for 30 min at room temperature and rinsed twice in a wash buffer. Next, 100 μl of 1:100 diluted phycoerythrin (PE)-conjugated goat antihuman IgG secondary antibody was added to the beads, and the beads were incubated for 30 min in the dark at room temperature and washed. The luminescence was read using a LABScreen™ 100 Luminex system (One Lambda Inc., Canoga, Park, CA, USA). Data were analysed using LABScreen analysis software HLA Fusion 2.0 (One Lambda), and a mean fluorescence intensity (MFI) of over 800 was considered positive. In this study cohort, none of the recipients had an MFI of over 2500. Therefore, we defined these patients as weakly sensitized recipients.

Diagnosis of graft rejection

Protocol biopsy after the operation was performed at postoperative day (POD) 14 and at least one more time

between 6 and 12 months after transplantation. Informed consent was obtained before the biopsy procedure. Protocol biopsy was not performed if the patients did not give their consent. Protocol biopsy in patients with complications, such as peri-renal fluid collection, bleeding tendency and wound infection, was postponed until those complications were resolved. In cases where rejection was suspected on clinical examination, such as when serum creatinine levels increased by 0.3 mg/dl above baseline or the patient had symptoms (e.g. oliguria or fever), episode biopsy was performed. Rejection or other pathological findings were diagnosed according to the Banff 07 criteria. Two or three core biopsy samples were obtained using a spring-loaded 16-gauge needle under ultrasound guidance. Diagnosis of rejection was made by the same two pathologists in all cases. The overall Banff diagnosis showed no differences between the two pathologists. The criteria of AMR in ABO-I were DSA positive and any of the following microvascular injuries: peritubular capillaritis (ptc > 0), glomerulitis (g > 0), thrombosis and transplant glomerulopathy (cg > 0).

Renal function

Renal allograft function was evaluated by the serum creatinine level (sCr) and the estimated glomerular filtration (eGFR). GFR was estimated using Cockcroft's formula. Glomerular filtration rate (GFR) was estimated based on the serum creatinine level, using the Filler equation and was expressed in ml/min/1.73 m^2.

Statistical analysis

All analyses were performed using the JMP software package (version 7.0 SAS Institute Inc., Cary, NC, USA). Data are expressed as means and standard deviation throughout the manuscript. Means of normally distributed values were compared by student's *t*-test. The McNemar chi-square test was used to compare proportions. Patient and graft survival rates were analysed by the Kaplan–Meier analysis. $P < 0.05$ was considered to be significant.

Results

Recipients with higher baseline titres showed significantly higher incidence of high rebound postoperatively

Before treatments for desensitization, 73 recipients showed low anti-ABO titres (1:32 or less) and 118 showed high titres (1:64 or higher) (Table 3). Anti-ABO titres rebounded to 1:64 or higher in 5 of 73 recipients with low baseline titres (5/73, 7%), while 16 recipients developed a high rebound in 118 recipients with high baseline titres (16/118, 14%). Recipients with higher baseline titres showed significantly higher incidence of high rebound (1:64 or higher) postoperatively ($P = 0.03$). The post-transplant rebound in anti-ABO titres was also analysed based on B cell-targeting therapy (splenectomy, rituximab, or both). There were no significant differences in the incidence of rebound among these different B cell-targeting therapies. Figure 2 shows patient and graft survival rates according to baseline titres using the Kaplan–Meier analysis. The graft survival of recipients with high baseline titres was slightly lower than those with low baseline titres, although not statistically significant.

Graft function after transplantation between recipients with low rebound (Group 1) and with high rebound (Group 2)

Serum creatinine levels (sCr) were significantly higher in Group 2 than in Group 1 at 2 weeks and 1 month after transplantation (1.48 ± 0.71 and 1.51 ± 0.72 mg/dl in Group 1 vs. 1.72 ± 1.03 and 1.76 ± 1.43 mg/dl in Group 2, respectively) ($P = 0.012$ at 2 weeks, $P = 0.02$ at 1 month) (Fig. 3). No significant differences in graft function were observed at 3 months, 6 months, 1 year, 3 years, 5 years or 10 years between the two groups.

No significant statistical differences in patient and graft survival rates between patients with low rebound (Group 1) and with high rebound (Group 2)

Figure 4 shows the differences in patient and graft survival rates between Group 1 and Group 2. Patient survival rates were 98% and 97% in Group 1, and 98% and 96% in Group 2, at 5 years and 10 years, respectively. Graft survival rates were 98%, 94% and 93% in Group 1 and 99%, 93% and 86% in Group 2 at 1, 5 and 10 years, respectively. Eleven recipients in Group 1 lost their grafts due to chronic rejection ($N = 6$), noncompliance ($N = 2$) and recurrence

Table 3. Pre-transplant ABO baseline titers in the low rebound (Group 1) and high rebound (Group 2).

	Low rebound (Group 1)	High rebound (Group 2)
Low baseline titers ($N = 73$)		
Total	68 (93%)	5 (7%)
Spx ($N = 17$)	15 (89%)	2 (11%)
Rit ($N = 54$)	51 (94%)	3 (6%)
Spx + Rit ($N = 2$)	2 (100%)	0 (0%)
High baseline titers ($N = 118$)		
Total	102 (86%)	16 (14%)
Spx ($N = 25$)	20 (80%)	5 (20%)
Rit ($N = 86$)	76 (89%)	10 (11%)
Spx + Rit ($N = 7$)	6 (86%)	1 (14%)

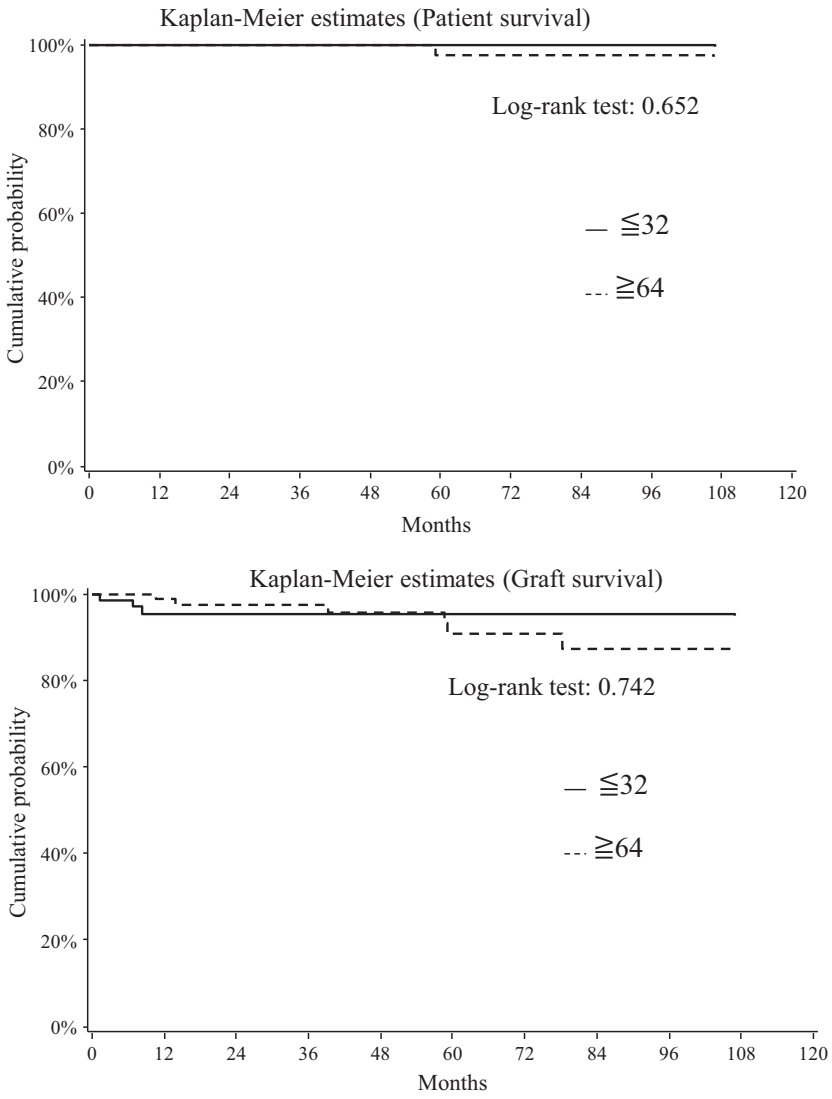

Figure 2 Patient and graft survival rate in patients with low baseline titers and with high baseline titers.

of original disease ($N = 3$; 1 IgA nephropathy and 2 focal glomerular sclerosis syndrome, FSGS). Three recipients in Group 2 lost their grafts due to unknown causes ($N = 1$), chronic rejection ($N = 1$) and noncompliance ($N = 1$). The graft survival of recipients with high rebound titres was slightly lower than those with low rebound titres, although not statistically significant.

No significant difference in any type of graft rejection between recipients with low rebound (Group 1) and high rebound (Group 2)

Table 4 compares the incidence rate of graft rejection of Groups 1 and 2. TMR occurred within 1 year in 13/170 (8%) recipients in Group 1 and 2 /21(10%) recipients in Group 2 ($P = 0.432$). AMR occurred within 1 year in 15/170 (9%) recipients in Group 1 and 2/21 (10%) in Group 2 ($P = 0.887$). Rejection-free status, including borderline change, was observed in 114 of 170 recipients in the low rebound group, Group 1, and in 15 of 21 recipients in the high rebound group, Group 2 (114/170, 67% in Group 1 vs. 15/21, 71% in Group 2, $P = 0.344$). There were no significant differences in any type of graft rejection between Groups 1 and 2. Further analyses of rejection rates according to B cell-targeting therapies, showed a significantly lower incidence of TMR in recipients treated with rituximab (Rit) in Group 1 ($P = 0.004$). In Group 2, there were no significant differences in incidence of TMR of AMR among recipients treated with different B cell-targeting therapy.

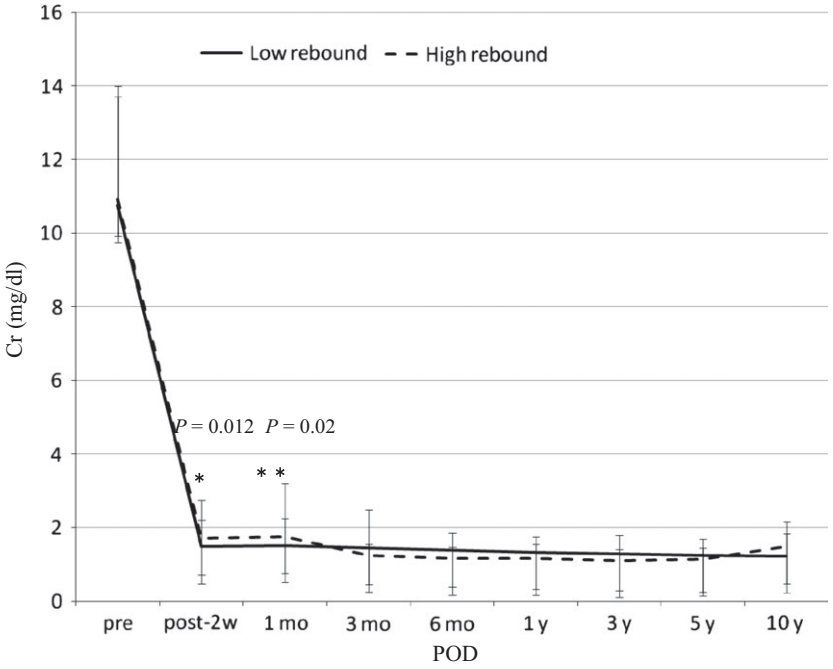

Figure 3 Graft function after Tx between recipients with low rebound titers (Group 1) and high rebound titers (Group 2).

Table 5 further analyses the relationship between DSA and the incidence of AMR. In Group 1, 28 of 170 recipients had DSA with 1510 ± 450 MFI (data, not shown), and 15 out of 28 recipients developed AMR. Two recipients in Group 1 experienced AMR due to the appearance of *de novo* anti-HLA antibody after transplantation. In Group 2, four of 21 recipients had DSA with 1890 ± 230 MFI (data, not shown), and two out of 4 recipients developed AMR. None experienced *de novo* AMR. On the basis of Table 5, all the AMR in this study were assumed to be caused by anti-HLA antibodies, not by antiblood type antibodies, although it is difficult to make a pathological differential diagnosis between them. Approximately 50% of the recipients in Groups 1 and 2 did not develop AMR despite the presence of DSA, because the ABO-I desensitized protocol was performed in all these recipients with mild sensitization status prior to transplantation.

There was no relevance between post-transplant anti-ABO rebounds and rejection or graft loss in Group 2 recipients

Table 6 and Figure 5 further analyse the outcome in Group 2 recipients. In these patients, the rebounds of anti-ABO titres were observed between 2 weeks and 1 year, which spontaneously decreased to less than 1:32 without any intervention. As shown in Table 6, among 21 recipients in Group 2, anti-ABO titres elevated to 1:128 or higher in 5 recipients, while the elevated titre was 1:64 in 16 recipients. None of the five recipients (NK, MY, AS, RK and KN) with rebounded titres greater than 1:128, including two (AS and RK) with titres of 1:512, experienced any rejection. Four recipients (TU, TT, NK and MO) had pre-DSA and two of them (TU and TT) showed AMR and TMR with DSA, which were resolved by antirejection therapy (steroid pulse and OKT3). Two patients (MO and TT) showed IFTA without active lesions. Three recipients (MT, TT and NI) in Group 2 lost their renal grafts. NI lost his graft 5 years after transplantation due to noncompliance to his medication. TT lost a graft due to mixed type rejection followed by chronic active antibody-mediated rejection 7 years after transplantation, though any treatments including ATG, and several steroid pulse therapies and plasma exchange were given. The cause of graft loss in MT has not been identified (no evidence of rejection).

Discussion

Immunosuppressive regimens with tacrolimus/MMF have yielded excellent results in ABO-I. The current survival rates for living-related ABO-I grafts have surpassed 90% at 10 years after transplantation [11]. Moreover, the preoperative, low-dose rituximab protocol alternative to splenectomy has also resulted in decreased morbidity as well as a lower incidence rate of acute and/or chronic antibody-mediated rejections over a 10-year period [16]. Currently, we have experienced very few cases of graft loss accompanied with rapid elevation of antiblood type antibody titres in ABO-I. In the present study, we investigated the

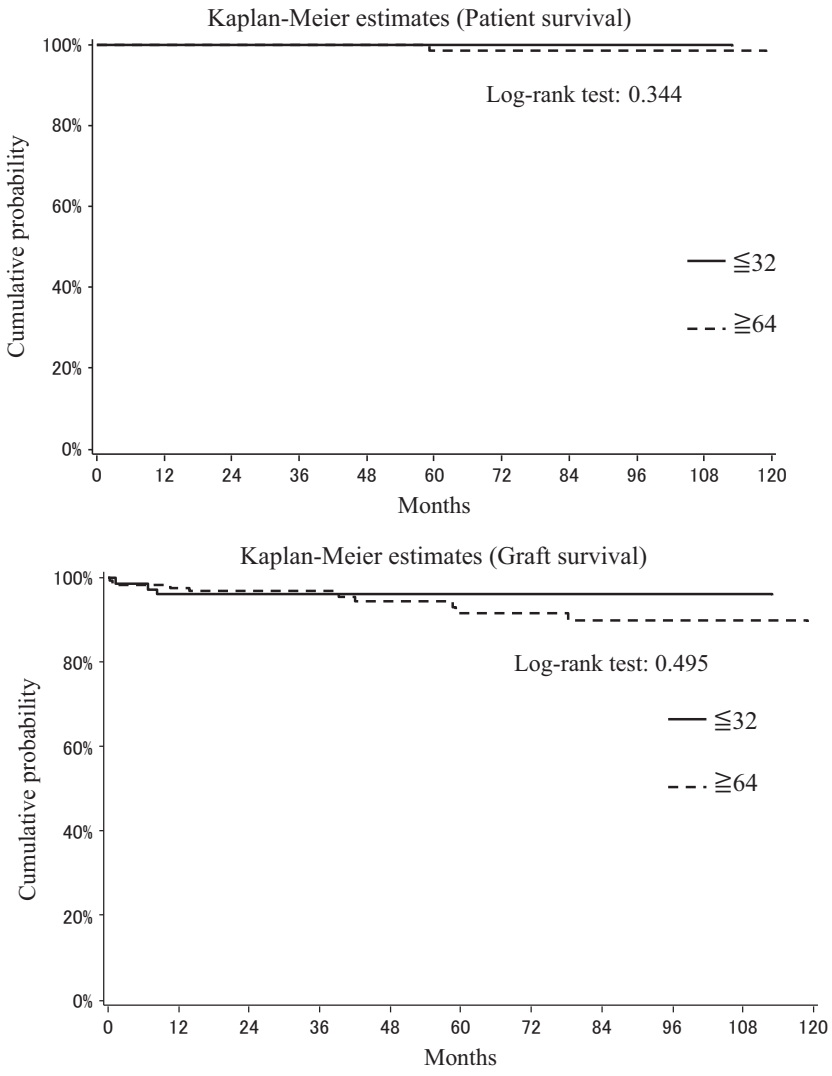

Figure 4 Patient and Graft survival rate between patients with low rebound titers (Group 1) and with high rebound titers (Group 2).

association between anti-ABO titres and the outcome of transplants in living-related ABO-I KTx.

Different from our previous report in which anti-ABO titres continued to rise in some CYA/azathioprine/splenectomy treated recipients [8], anti-ABO titres in patients treated with a tacrolimus/MMF-based regimen spontaneously decreased to normal range within 1 year without any intervention. We also analysed the changes in anti-ABO titres and the pathological findings according to B cell-targeting therapies (splenectomy or rituximab). As for the changes in anti-ABO titres, there was no significant difference in postoperative titres between recipients treated with splenectomy and rituximab. However, the pathological analyses revealed a significantly lower incidence of TMR in recipients treated with rituximab versus splenectomy. The lower incidence of TMR may be attributed to inhibition of T cell–B cell interactions by rituximab, as previously reported in patients with severe SLE [17]. Rituximab affects most mature B cell subsets with no effect on plasma cells in the spleen or in the second lymphoid organs. It changes the phenotype of B cell populations at the time of immunological reset after complete peripheral depletion [18]. Moreover, it has recently been suggested that rituximab may also cause inactivation of T cells indirectly [19]. The effects of rituximab are likely to arise from the impairment of the B cell regulation of T cells such as CD8 recall response [20] and CD4 activation [21].

Previously, we observed a significant difference in *de novo* anti-HLA antibody production between the rituximab and splenectomy groups [16]. However, we have not observed a similar suppression of anti-AB titres between these two groups because of the difference in characteristics

Table 4. Incidence rate of graft rejection between recipients with low rebound titers (Group 1) and high rebound titers (Group 2) within 1 year after Tx.

	TMR	AMR	IFTA
Group 1: Low rebound ≦32 (*N* = 170)	13 (8%)	15 (9%)	29 (17%)
Spx (*N* = 35)	12 (34%)*	4 (11%)	5 (14%)
Rit (*N* = 127)	1 (1%)*	11 (9%)	22 (17%)
Spx + Rit (*N* = 8)	0 (0%)	0 (0%)	2 (25%)
Group 2: High rebound ≧64 (*N* = 21)	2 (10%)	2 (10%)	2 (10%)
Spx (*N* = 7)	1 (14%)	1 (14%)	1 (14%)
Rit (*N* = 13)	1 (8%)	1 (8%)	1 (8%)
Spx + Rit (*N* = l)	0 (0%)	0 (0%)	0 (0%)
P value	0.432	0.887	0.211

*$P = 0.004$.

Table 5. The relationship between DSA and AMR in Groups 1 and 2.

		DSA	
	AMR	Preformed DSA	*de novo* DSA
Group 1: Low rebound ≦32 (*N* = 170)	15	13	2
Group 2: High rebound ≧64 (*N* = 21)	2	2	0

of B cell lineage such as B-1a, B-1b and B2 that are capable of antiblood type antibody and anti-HLA antibody. Bedside observation shows that rituximab alone does not decrease the antiblood AB titres (data, not shown).

The Tacrolimus/MMF combination is likely to have a continuous suppressive effect on B cell lineage that is capable of producing antiblood type antibodies [22]. Irei, *et al.* reported the suppression of segregation to $CD5^+$ B-1a cells by calcineurin inhibitors in small animal models. They also found that the proportion of B-1a cells in the peripheral blood was significantly reduced in patients persistently taking either tacrolimus or cyclosporine in their preliminary study.

The association between baseline or rebounded titres and their pathological findings has recently been reported both by Korean and Johns Hopkins groups [23,24]. Chung *et al.* [23] reported a higher tendency of antibody rebound and a higher risk for acute rejection in recipients with a baseline titre higher than 1:512. The definition of high titre was different from ours (ours, 1:64 and the Korean, 1:512). As we reported previously [8], all recipients with a rebound greater than 1:64 lost their grafts postoperatively. After those observations, we defined the high antiblood type antibody titre as a titre 1:64 or higher. Using the Korean's criteria (1:512 or more as a high titre) in the present study, 1 (TT) of 4 (MA, NK, AS and TT) recipients with more than 1:512 at baseline experienced antibody-mediated rejection, and none of the recipients (NK, MY, AS, RK and KN) with more than 1:256 or 1:512 rebounded titres experienced any type of acute rejection. Even in recipients with high titres above 1:512, a clear association between high titre and incidence of acute rejection was not observed in our study. As a consensus of the definition of AMR in ABO-I KTx has not been made, the difference between our report and that of the Korean group may be due to a difference in the pathological diagnosis of AMR in ABO-I. Our criteria for AMR have been based on microvascular injuries: peritubular capillaritis (ptc > 0), glomerulitis (g > 0), thrombosis and transplant glomerulopathy (cg > 0) rather than C4d deposition. The Korean group did not present detailed criteria for AMR in ABO-I [23].

The apparent resistance of a vascularized graft to humoral rejection despite the presence of antibodies in the recipient's body is termed 'accommodation'. The mechanisms for accommodation are still unknown, although several have been proposed [25]. One such is a decrease in the antibody–antigen interaction that gives rise to antibody-mediated rejection. Decreased antibody–antigen interaction could result from a change in the repertoire or in the level of antibodies in the circulation of the recipient or a change in the expression status of the target antigens [26]. An alternative explanation for accommodation is that with continued stimulation of endothelial cells by antibodies or complement components circulating in the recipient, the susceptibility of these cells to injury decreases. Continued stimulation of endothelial cells by endotoxin or IL-1 causes the cells to change in a way that their response to re-stimulation is diminished. We also observed a decrease of blood type antigens on endothelial cells in recipients with excellent function after ABO-I [27]. In contrast, endothelial mixed-chimerism with recipient-derived antigens can be observed on endothelial cells from poor-functioning renal grafts due to irreversible, severe damage such as HLA antibody-mediated rejection or CNI toxicity after ABO-I [28].

Tobian *et al.* [24] of the Johns Hopkins group also reported that the incidence of AMR was significantly higher in recipients with high post-transplant titres of more than 1:64. In their report, they hypothesized that post-transplant plasmapheresis is helpful in preventing rejection, but they also noted that the relationship between the exact mechanism of the antibody rebound and accommodation remains to be elucidated, because many recipients with high rebound also did not experience rejection in their study.

A couple of limitations should be noted when one compares our results with those of other centres. First of all, although the difference between results may not be significant, the method of detecting antiblood type antibodies is different at each centre. Tobian, *et al.* at Johns Hopkins

Table 6. Anti-blood type antibodies and the biopsy in Group 2 recipients.

				Anti-blood type antibody						
Name	Gender	B cell strategy	Pre-DSA	Base	2 weeks	1 month	3 months	6 months	1 year	Pathological findings (date for graft-biopsy)
MO	F	Rit	+	256	64	64	64	32	16	IFTA (1 month), 2nd Tx
MT	M	Spx	–	256	64	32	–	–	16	Graft loss
YO	M	Spx	–	256	64	32	32	32	16	No AR (6 months)
MA	F	Spx	–	512	64	32	32	64	–	No AR (2 weeks, ly)
MY	M	Spx	–	32	32	–	–	–	64	No AR (2 weeks, 2 months)
TI	M	Spx	–	128	64	–	32	32	16	AVR (2 weeks), No AR (3 months)
NK	M	Spx	+	512	64	64	128	256	64	No AR (1 month, 6 months)
MY	M	Rit	–	256	64	128	256	128	–	No AR (2 weeks)
AS	F	Rit, Spx	–	1024	256	512	256	128	128	No AR (2 weeks, 6 months)
TU	M	Spx	+	16	64	32	–	16	–	TMR (2 weeks), AMR (1 month)
RK	M	Rit	–	8	512	512	8	4	4	No AR (1 month)
JK	F	Rit	–	256	64	–	64	64	16	No AR (2 weeks, 6 months)
TT	F	Rit	+	1024	64	–	8	–	8	TMR (3 weeks), AMR (3 months), IFTA (1 year)
KS	M	Rit	–	16	4	16	64	–	64	No AR (3 months)
MK	F	Rit	–	256	4	–	64	–	64	No AR (2 weeks, 3 months, 9 months, 1 year)
TY	M	Rit	–	64	8	64	–	64	64	No AR (2 weeks, 1 year)
TS	M	Rit	–	64	4	64	–	–	64	No AR (3 weeks)
NY	F	Rit	–	64	8	–	64	64	64	No AR (2 weeks, 1 year)
YI	M	Rit	–	32	–	64 (IgM)	32	4	64	No AR (1 year)
NI	F	Rit	–	64	–	32	64	64	–	No AR (3 weeks)
KN	M	Rit	–	128	32	256	128	64	32	No AR (3 weeks)

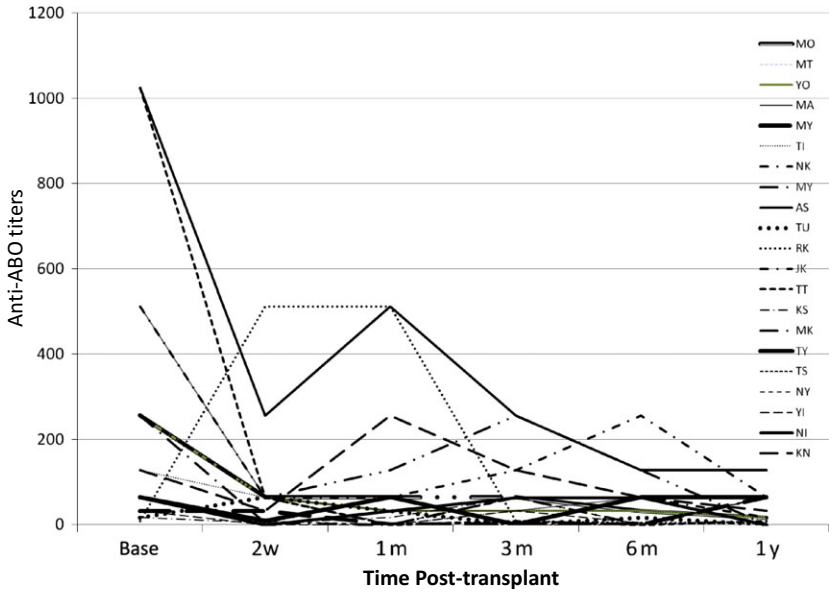

Figure 5 Change in anti-blood type antibodies before and after Tx in recipients with high rebound titers, more than 1:64.(*N* = 21).

Hospital adopted the Marsh scoring system to monitor antibodies simultaneously with the classical agglutination assay. We mainly adopted the agglutination and ELISA assays to monitor antibodies, as reported previously [29]. Secondly, there has been a lack of understanding on the effect of antiblood antibody subtype IgG and/or IgM on renal allografts. Blood type antibodies in this study were mainly IgG antibodies, except for one case, YI. This patient showed IgM elevation to 1:64 at 1 month postoperatively, followed by the elevation of IgG to 1:64 at 1 year. Severe colitis immediately after transplantation may have triggered the production of IgM and subsequently IgG antibodies in

this patient. Fortunately, he did not develop antibody-mediated rejection despite these elevated antiblood type antibodies. Thirdly, there is no consensus on the diagnosis of AMR in ABO-I. Further studies are necessary to establish the optimal diagnostic criteria for AMR.

Since the application of the Tacrolimus and MMF combination in 2000, we have rarely experienced AMR (clinical or subclinical) accompanied with rebounded high anti-ABO titres. In the present study, among 21 recipients with high postoperative rebounds of over 1:64, only 2 recipients showed subclinical AMR within 3 months, in spite of not receiving prophylactic treatment. As shown in Table 4, the incidence rate of AMR and TMR was almost 10%, regardless of the rebounded titres. Although the decision of treatment should be made on a case-by-case basis, we conclude that B cell-targeting treatment for postoperatively rebounded anti-ABO antibodies is not necessary in the majority of cases.

Authorship

HI: designed the research/study, performed the research/study and wrote the paper. TK: designed the research/study. TS: pathological work. TN: wrote the paper. KT: contributed important reagents.

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