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Different sensitivity of rituximab-treatment to B-cells between ABO-incompatible kidney and liver transplantation

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ABSTRACT

A desensitization protocol with rituximab is currently widely used for kidney transplantation (KT) and liver transplantation (LT) across the ABO blood group-incompatible (ABO-I) barrier. However, it remains to be elucidated whether rituximab is equally effective for B-cell and T-cell immune responses in both KT and LT recipients. To clarify these effects of rituximab, we enrolled 46 KT and 77 LT recipients in this study. The proportion of peripheral blood B-cells was determined at the perioperative period. T-cell responses to allostimulation were evaluated by a mixed lymphocyte reaction (MLR) assay. One week after rituximab administration, peripheral B-cells became undetectable in ABO-I KT recipients but remained detectable in some of the ABO-I LT recipients; B-cells were undetectable in both groups by week 2. B-cells remained below the detection limit throughout the first year in the ABO-I KT recipients, whereas they reappeared in the periphery after 6 months in the ABO-I LT recipients. There were no significant differences in alloreactive T-cell responses based on MLR analyses between ABO-I and ABO-compatible groups. This study indicates that rituximab has differing B-cell sensitivity between KT and LT recipients and a minimal effect on the alloreactive T-cell responses in KT and LT recipients.

1. Introduction

The use of ABO-incompatible (ABO-I) donor organs is a possible solution for the shortage of donor organs for transplantation; however, naturally occurring antibodies (Abs) against blood group A or B (A/B) carbohydrate determinants in sera are a major impediment to achieving successful transplantation. Plasmapheresis or plasma exchange, splenectomy, and/or anti-B-cell immunosuppressant treatment in the recipients are widely adopted strategies to remove pathologic anti-A and anti-B Abs and prevent Ab-mediated rejection (AMR) of ABO-I organ grafts [1]. Using these modalities, ABO-I kidney transplants (KTs) have achieved graft and patient survivals similar to that seen in ABO-compatible (ABO-C) transplants [2]. Among those, the prophylactic use of rituximab, a monoclonal chimeric human-murine anti-CD20 Ab that depletes B-cells by complement-dependent cytotoxicity (CDC), Ab-dependent cell-mediated cytotoxicity (ADCC), and stimulation of apoptosis [3–5], is currently indispensable to achieving successful ABO-I KT [2]. Treatment with rituximab has also been applied in adult ABO-I living-donor liver transplant (LT) and improved outcomes to the level comparable to ABO-C LT [6].

At our institute, both ABO-I KT and LT recipients were preconditioned prior to surgery with a common desensitization protocol that consisted of a single dose of rituximab and subsequent daily

Abbreviations: Ab, antibody; ABO-C, ABO-blood-type compatible; ABO-I, ABO-blood-type incompatible; ADCC, antibody-dependent cell-mediated cytotoxicity; AMR, antibody-mediated rejection; AR, acute rejection; BMI, body mass index; CDC, complement-dependent cytotoxicity; CFSE, carboxyfluorescein diacetate succinimidyl ester; CMV, cytomegalovirus; CNI, calcineurin inhibitor; CsA, cyclosporine A; dnDSA, *de novo* donor specific anti-human leukocyte antigen antibody; DSA, donor-specific anti-human leukocyte antigen antibody; FCM, flow cytometry; FITC, fluorescein isothiocyanate; HLA, human leukocyte antigen; KT, kidney transplant; LT, liver transplant; mAb, monoclonal antibody; MELD, model for end-stage liver disease; MFI, mean fluorescence intensity; MLR, mixed lymphocyte reaction; MMF, mycophenolate mofetil; MP, methylprednisolone; NK, natural killer; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; SI, stimulation index; TAC, tacrolimus.

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internal use of calcineurin inhibitor (CNI) and mycophenolate mofetil (MMF). This provided a unique opportunity to study whether the susceptibility to rituximab might be different between patients with kidney and liver failure, who had different metabolic, pharmacokinetic, and complement activity etiologies. In addition to B-cell depletion, treatment with rituximab might also influence T-cell responses to alloantigens since B-cells are effective antigen-presenting cells capable of activating donor-specific T-cells within peripheral lymph nodes [7,8]. B-cell depletion via rituximab therapy in patients with end-stage renal failure had minimal impact on T-cell function and cytokine release [9], and a recent randomized double-blind placebo-controlled study revealed that B-cell depletion by rituximab in KT recipients did not affect T-cell phenotype and function after transplantation [10]. On the other hand, it was suggested that the cytokine release syndrome caused by rituximab may enhance T-cell activation, thereby increasing acute rejection (AR) rates [11]. A recent study demonstrated that rituximab can modulate the immune response by inducing cytokine secretion, especially that of interleukin 10 and macrophage inflammatory protein 1 beta [12]. Such incongruous opinions prompt us to investigate the influence of rituximab on T-cells by comparing alloimmune responses between ABO-C and ABO-I KT/LT recipients. Hence, the objective of this study was to elucidate whether rituximab is equally effective in abrogating B-cell and even T-cell immune responses in KT and LT recipients.

2. Patients and methods

2.1. KT recipients

Between October 2006 and March 2013, 65 patients underwent living-donor KT at Hiroshima University Hospital. Of these, 19 patients were excluded from the study because of the presence of donor-specific anti-human leukocyte antigen Abs (DSAs) at the time of KT with/without usage of bortezomib (n = 5) [13,14], other organ transplantation (liver n = 1, lung n = 1), tertiary transplantation (n = 1), or incomplete immune monitoring data caused by limited volume of stored lymphocytes from donors for in vitro mixed lymphocyte reaction (MLR) assays (n = 11). The remaining 46 patients (18 ABO-I recipients and 28 ABO-C recipients) were enrolled in this study. The following information was collected at the time of the transplant: age, gender, body mass index (BMI), human leukocyte antigen (HLA) mismatch, relationship, primary disease, and dialysis period.

2.2. LT recipients

Between April 2007 and March 2013, 105 patients underwent living-donor LT at Hiroshima University Hospital. Of these, 28 patients were excluded from the study because of re-transplantation (n = 4), DSAs at the time of LT (n = 1), KT that had been performed before LT (n = 1), usage of bortezomib after LT (n = 1), or incomplete immune monitoring data caused by limited volume of stored lymphocytes from donors for in vitro MLR assays (n = 21). The remaining 77 patients (14 ABO-I recipients and 63 ABO-C recipients) were enrolled in this study. The following information was collected at the time of the transplant: age, gender, BMI, HLA mismatch, relationship, splenectomy, primary disease, Child-Pugh score, and model for end-stage liver disease (MELD) score.

2.3. Desensitization protocol

This study was conducted with informed consent using a protocol approved by the institutional review board of the Hiroshima University Hospital (No. 625). The recipients of ABO-I KT and LT were treated with a common desensitization regimen. At 2 weeks before transplantation, a single dose of rituximab (375 mg/m^2 body surface) was administered to recipients. Subsequently, all subjects received a CNI, i.e., tacrolimus (TAC, target trough level: 5–10 ng/mL) or cyclosporine A (CsA, target trough level: 80–100 ng/mL) and MMF (10–20 mg/kg/day) and underwent 0–5 sessions of plasma exchange or double-filtration plasmapheresis to decrease anti-blood group isoagglutinin titers at least 16-fold before surgery.

2.4. KT immunosuppression protocol

The basic immunosuppressive regimen after ABO-I KT was the same as that after ABO-C KT. Basiliximab was administered at a dose of 20 mg/day at the time of transplantation and on postoperative day 4. The regimen after transplantation comprised CsA, MMF, and methylprednisolone (MP) with gradually tapering doses. The trough whole blood levels of CsA were maintained between 200 and 250 ng/mL in the first few postoperative weeks and between 150 and 200 ng/mL thereafter.

2.5. LT immunosuppression protocol

The basic immunosuppressive regimen after ABO-C LT comprised TAC and MP at gradually tapering doses. Trough whole blood levels of TAC were maintained between 8 and 15 ng/mL in the first few postoperative weeks and between 5 and 10 ng/mL thereafter. The regimen after ABO-I LT comprised TAC, MMF, and MP. The trough whole blood levels of TAC were the same as those in ABO-C LT recipients.

2.6. B-cell and natural killer (NK) cell analyses

In ABO-I KT and LT recipients, the proportion of peripheral blood B-cell and NK-cell subsets was determined at pre-desensitization; immediately before transplantation; 1 and 2 weeks after rituximab administration; and 1, 3, 6, 9, and 12 months after transplantation.

For B-cell phenotyping, peripheral blood mononuclear cells (PBMCs) were stained with fluorescein isothiocyanate (FITC)-conjugated anti-IgM (BD Pharmingen, San Diego, CA, USA) and PE-conjugated anti-CD19 (BD Pharmingen) monoclonal antibodies (mAbs). B-cells were defined as lymphocytes with both IgM- and CD19-positive phenotypes. For phenotyping NK cells, PBMCs were stained with FITC-conjugated anti-CD3 (BD Pharmingen) and PE-conjugated anti-CD56 (BD Pharmingen) mAbs. NK cells were defined as lymphocytes with CD3-negative and CD56-positive phenotypes. Dead cells were excluded from the analysis by light scattering and/or propidium iodide staining. Flow cytometric (FCM) analyses were performed on a FACSCalibur® dual-laser cytometer (BD Biosciences, Mountain View, CA, USA). Representative dot plots for B-cells and NK cells are shown in Supplemental Fig. 1.

2.7. Immune monitoring by in vitro MLR assays

To evaluate the immune reactivity of KT and LT recipients, T-cell responses to allostimulation were evaluated by an MLR assay using an intracellular carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling technique during the preoperative period (before desensitization in the case of ABO-I recipients) and 0.5, 1, 3, 6, and 12 months after transplantation. The CFSE-MLR allows quantification of cell proliferation in response to allogeneic stimuli and simultaneous determination of proliferating cell phenotypes by using multiparameter FCM analysis. T-cell proliferation was visualized by twofold serial dilutions of the fluorescence intensity

of CFSE. $CD4^+$ and $CD8^+$ T-cell proliferation and stimulation index (SI) were quantified using a previously described method [15,16].

2.8. Antibody detection

Anti-HLA single antigen reactivity was detected on a Luminex platform (LABScan 100 flow analyzer, Luminex Corporation, Austin, TX, USA) according to the manufacturer's protocol using LABScreen Single Antigen assays. The results were recorded as mean fluorescence intensity (MFI). MFI values greater than 1000 were considered positive. *De novo* DSAs (dnDSAs) were defined as HLA-A, B, C, DRB1, or DQB1 Abs detected against the donor HLA that were not present pre-transplant. The anti-blood type isoagglutinin titers for IgM and IgG were serially measured as previously reported [17].

2.9. Definitions and other laboratory data

Diagnosis of AR was based on Banff criteria in episode biopsies. Biopsies were performed when laboratory tests showed abnormal findings. A bacterial, viral, or fungal infection was defined as positive if clinical signs of acute infection were found and serologic markers or culture were positive. Cytomegalovirus (CMV) antigenemia-positive was defined as the detection of more than 3/50,000 CMVpp65-positive cells. Neutropenia was defined as a neutrophil count of 1000 or fewer neutrophils per microliter of blood. Clinical and laboratory data were extracted from patient medical charts.

2.10. Statistical analysis

Quantitative variables were expressed as mean ± standard error or as median and range. Student's *t*-test, Mann–Whitney *U*-test, chi-squared test, and Fischer's exact test were used to compare variables between the two groups. Equality of variance was examined using an *F*-test, and the result was corrected by the Bonferroni method. Kaplan–Meier analyses were used to compare time-to-event variables. Differences among the curves were examined using a log-rank test. *P*-values below 0.05 were considered statistically significant.

The impact of ABO-I transplantation on T-cell response, graft survival, AR, infection, neutropenia, and dnDSA after transplantation was retrospectively evaluated by a 1:1 (KT) or 1:2 (LT) match using propensity scores to overcome bias due to the different distribution of covariates for the ABO-I and ABO-C groups. The calculated propensity scores indicate the conditional probability that a subject belongs to the ABO-I or ABO-C transplantation groups. Analyses were performed with an SPSS R-menu for propensity score matching in IBM SPSS statistics 22 using R statistical software version R2.15.2. For KT, propensity scores were estimated using logistic regression with gender; primary disease group; HLA mismatch count in A, B, and DR; relationship; and treatment before transplantation as dichotomous covariates and age, BMI, and dialysis periods as continuous covariates. For LT, propensity scores were estimated using logistic regression with gender; primary disease group; HLA mismatch count in A, B, and DR; splenectomy; and relationship as dichotomous covariates and age, BMI,

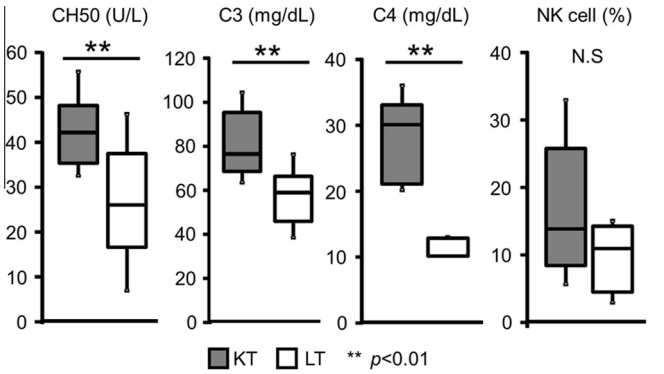

Fig. 2. Serum complement levels and proportion of peripheral blood NK cells of kidney transplant (KT) and liver transplant (LT) recipients before desensitization. The serum CH50, C3, and C4 levels of ABO-I LT recipients before administration of rituximab were significantly lower than those of ABO-I KT recipients, whereas the proportion of peripheral blood NK cells was not significantly different between these two groups. The box plot indicates the 25th, 50th, and 75th percentiles, and the extended bars represent the 10th through 90th percentiles. The gray and white boxes indicate KT and LT recipients, respectively. The Mann–Whitney *U*-test was used to test differences between KT and LT values.

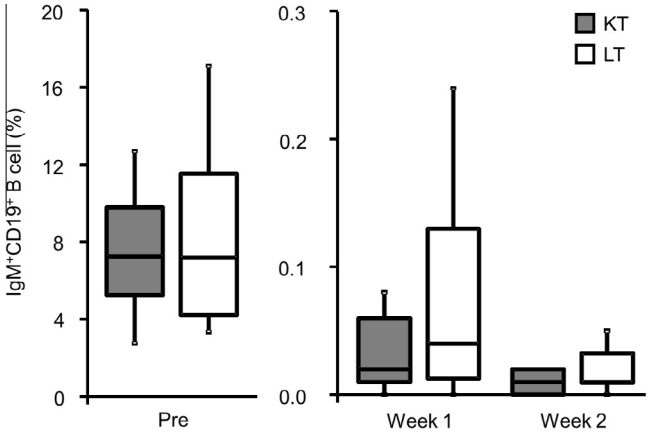

Fig. 1. Kinetics of the proportion of peripheral blood IgM^+ $CD19^+$ B-cell subsets in kidney transplant (KT) and liver transplant (LT) recipients during desensitization. The proportion of peripheral blood IgM^+ $CD19^+$ B-cells in all of the ABO-I KT recipients decreased below 0.1% at 1 week after rituximab administration. In contrast, the proportion in some of the ABO-I LT recipients remained above 0.1% until 2 weeks after rituximab administration. The box plot indicates the 25th, 50th, and 75th percentiles, and the extended bars represent the 10th through 90th percentiles. The gray and white boxes indicate KT and LT recipients, respectively. The Mann–Whitney *U*-test was used to test differences between KT and LT values.

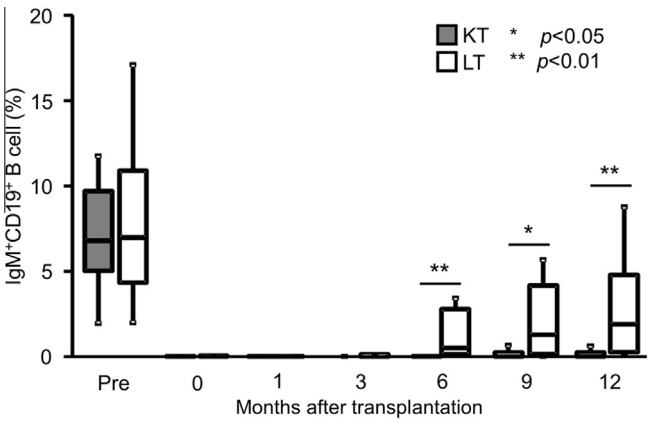

Fig. 3. Kinetics of the proportion of peripheral blood IgM^+ $CD19^+$ B-cell subsets in kidney transplant (KT) and liver transplant (LT) recipients after transplantation. The proportions of IgM+ CD19+ B-cells in the peripheral blood remained below 0.1% throughout the first year in the ABO-I KT recipients, whereas the cells reappeared in the peripheral blood after 6 months in the ABO-I LT recipients. The box plot indicates the 25th, 50th, and 75th percentiles, and the extended bars represent the 10th through 90th percentiles. The gray and white boxes indicate KT and LT recipients, respectively. The Mann–Whitney *U*-test was used to test differences between KT and LT values.

Child-Pugh score, and MELD score as continuous covariates. After estimation of the propensity scores, 1:1 (KT) or 1:2 (LT) nearest-neighbor matching was performed. The c-index values were 0.778 and 0.878, respectively.

3. Results

3.1. Proportion of peripheral blood IgM$^+$ CD19$^+$ B-cells and CD3- CD56 + NK cells in ABO-I KT and LT recipients

The proportion of peripheral blood IgM$^+$ CD19$^+$ B-cells in all of the ABO-I KT recipients decreased below 0.1% at 1 week after administration of rituximab. In contrast, that in some of the ABO-I LT recipients remained above 0.1% at this time point but eventually decreased below 0.1% in by week 2 (Fig. 1). Since CDC and ADCC should mediate the B-cell-depleting effect of rituximab, rituximab susceptibility in patients might be associated with their complement and/or immunocyte activities developing opsonization. The serum CH50, C3, and C4 levels of ABO-I LT recipients before administration of rituximab were significantly lower than those of ABO-I KT recipients ($p < 0.01$), whereas the proportion of peripheral blood NK cells was not significantly different between these two groups (Fig. 2). Thus, the slower tempo of B-cell depletion by rituximab in the LT recipients might reflect their lower complement activities. The proportions of IgM$^+$ CD19$^+$ B-cells in the peripheral blood remained below 0.1% throughout the first year in the ABO-I KT recipients, whereas IgM$^+$ CD19$^+$ B-cells reappeared in the periphery after 6 months in the ABO-I LT recipients (Fig. 3). In these patients, IgM$^+$ CD27$^+$ memory B-cells continued to be suppressed during the first year after transplantation (Supplemental Fig. 2). The faster tempo of B-cell replenishment in the LT recipients might reflect their lower dosage of immunosuppressants as compared to that of KT recipients. Consistently, the mean MMF and MP doses in ABO-I LT recipients were significantly lower than those in ABO-I KT recipients during the first year after transplantation, except for MP doses at 1 month after transplantation (Supplemental Fig. 3). There was no difference in the proportion of peripheral blood NK cell subsets between KT and LT recipients at each time point (Supplemental Fig. 4).

3.2. Serum immunoglobulin levels and anti-blood group isoagglutinin titers after KT and LT

Since the use of rituximab may cause hypoglobulinemia, serum levels of IgM and IgG after KT and LT were determined. In both KT and LT, serum levels of IgM in ABO-I groups were significantly lower than those in ABO-C groups at multiple time points within 1 year after transplantation, probably reflecting persistent B-cell dysfunction after the single administration of rituximab (Fig. 4). In contrast, serum IgG levels did not differ significantly between ABO-I and ABO-C groups in either KT or LT recipients. This might be explained by the fact that rituximab targets CD20$^+$ B-cells, which secrete IgM, but not plasma cells, which secrete IgG [18].

Anti-blood group isoagglutinin titers decreased below 8-fold at the time of transplantation and were maintained at low levels during the observation period after both KT and LT (Fig. 5).

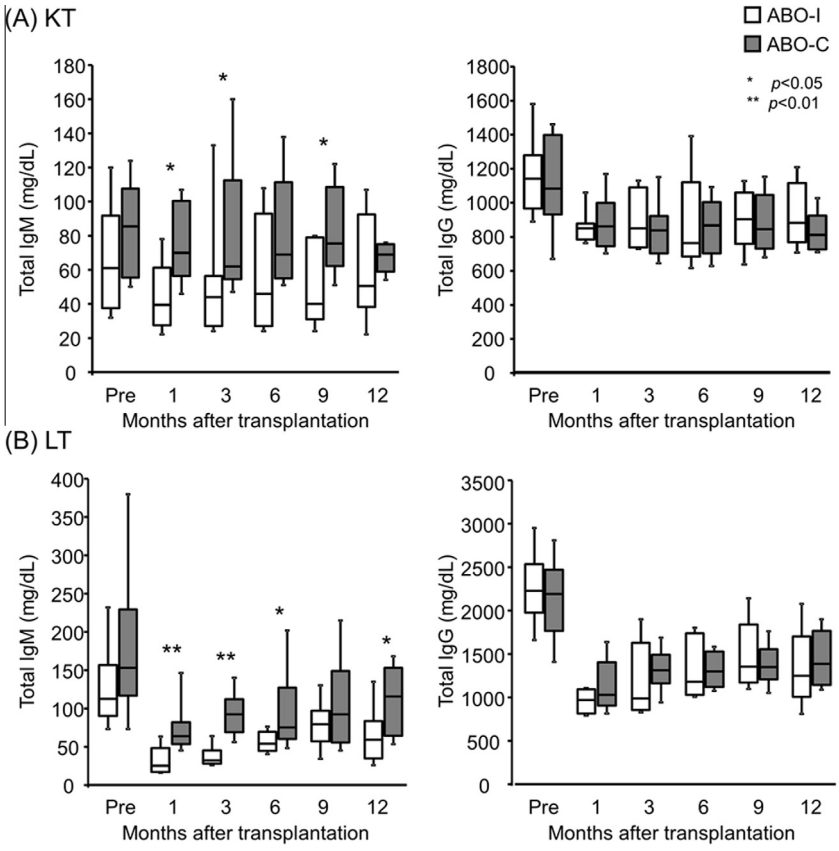

Fig. 4. Serum immunoglobulin levels in the ABO-blood type-incompatible (ABO-I) and ABO-blood type-compatible (ABO-C) groups during the first year after (A) kidney transplantation (KT) and (B) liver transplantation (LT). In both KT and LT recipients, serum levels of IgM in the ABO-I groups were significantly lower than those in the ABO-C groups at multiple time points within 1 year after transplantation. In contrast, serum IgG levels did not differ significantly between the ABO-I and ABO-C groups in either KT (A) or LT (B). The box plot indicates the 25th, 50th, and 75th percentiles, and the extended bars represent the 10th through 90th percentiles. The white and gray boxes indicate ABO-I and ABO-C groups, respectively. The Mann–Whitney *U*-test was used to test differences between ABO-I and ABO-C groups.

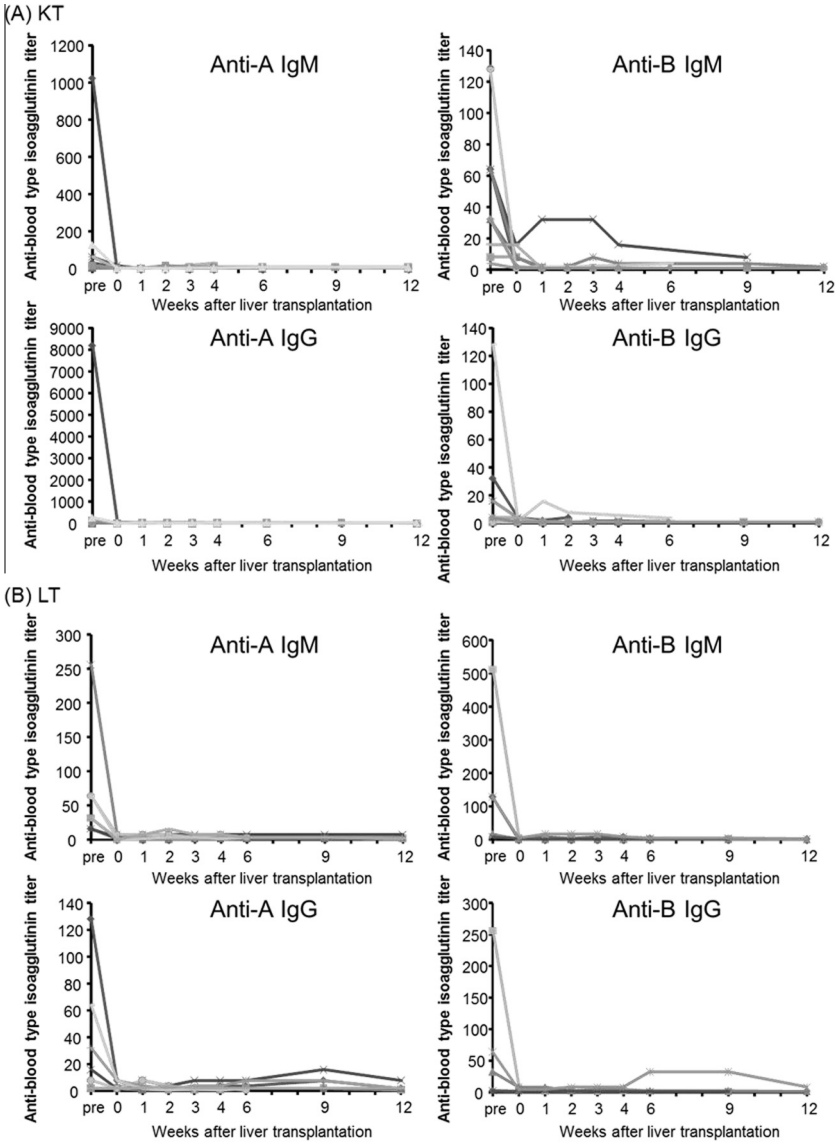

Fig. 5. Anti-blood group isoagglutinin titers after (A) kidney transplantation (KT) and (B) liver transplantation (LT). Anti-blood group isoagglutinin titers decreased below 8-fold at the time of transplantation and remained at low levels during the observation period after KT and LT. The lines indicate anti-A/B IgM/G isoagglutinin titers for each patient.

3.3. T-cell immune responses to allostimulation in KT and LT recipients

To evaluate the T-cell immune status of recipients, we performed a serial MLR assay using a CFSE-labeling technique. Since T-cell immunity is likely influenced by various factors such as primary disease and HLA matching, ABO-I and ABO-C recipients in KT/LT were matched by propensity scores to overcome bias due to the different distribution of covariates for the groups. The characteristics of these recipients after matching are listed in Tables 1A and 1B, indicating that there were no significant differences in the characteristics between the two groups. During the first year after transplant, the SIs for $CD4^+$ and $CD8^+$ T-cells in response to donor-type stimuli in the ABO-I group tended to be lower than those in the ABO-C group, although the difference was not statistically significant (Fig. 6). The SIs for $CD4^+$ and $CD8^+$ T-cells in response to third-party stimuli in the ABO-I group displayed a similar trend. The SIs for $CD8^+$ T-cells in response to donor-type and third-party stimuli at 1 year after transplantation were lower than those before transplantation. Of note, the variances of the SIs for anti-donor $CD4^+$ T-cells in the ABO-I groups were generally smaller than those in the ABO-C groups, at least at the early phase after surgery, suggesting that the desensitization regimen in the ABO-I groups might decrease excessive immune responses in immunological high-responder patients.

3.4. AR, infection, neutropenia, dnDSA, and graft survival

Table 2 shows the comparison of ABO-I and ABO-C transplant recipients matched by propensity scores for the following incidences: AR; CMV antigenemia positivity; infections such as varicella zoster virus, fungal infections, and blood stream infections; neutropenia; and dnDSA. There were no significant differences in these incidences between the two groups, except for the CMV antigenemia-positive rates. The 5-year graft survival rates of ABO-I and ABO-C LT were 71.4% and 79.6%, respectively, whereas those of ABO-I and ABO-C KT were both 100%, indicating no differences in graft survival between ABO-I and ABO-C.

Table 1A
Patient characteristics after propensity score matching (kidney transplantation).

	Incompatible N = 18	Compatible N = 18	P value
Age (years: median, range)	52.0 (30.0–71.0)	47.5 (28.0–66.0)	0.339
BMI	20.95 (17.18–31.52)	21.81 (18.14–29.87)	0.481
Dialysis period (years: median, range)	0.52 (0–12.05)	1.39 (0–8.53)	0.443
Gender			1.000
Male	10 (55.6%)	11 (61.1%)	
Female	8 (44.4%)	7 (38.9%)	
Primary disease			0.603
IgA	4 (22.2%)	6 (33.3%)	
DM	3 (16.7%)	3 (16.7%)	
CGN	4 (22.2%)	1 (5.6%)	
Others	7 (38.9%)	8 (44.4%)	
Pre-transplant treatment			0.427
Preemptive	6 (33.3%)	5 (27.8%)	
HD	8 (44.4%)	4 (22.2%)	
PD	2 (11.1%)	6 (33.3%)	
HD and PD	2 (11.1%)	3 (16.7%)	
A mismatch			0.780
0	6 (33.3%)	8 (44.4%)	
1	6 (33.3%)	6 (33.3%)	
2	6 (33.3%)	4 (22.2%)	
B mismatch			0.793
0	1(5.6%)	2 (11.1%)	
1	8 (44.4%)	9 (50.0%)	
2	9 (50.0%)	7 (38.9%)	
DR mismatch			0.725
0	1 (5.6%)	1 (5.6%)	
1	10 (55.6%)	13 (72.2%)	
2	7 (38.9%)	4 (22.2%)	
Relationship			0.505
Unrelated	10 (55.6%)	7 (38.9%)	
Related	8 (44.4%)	11 (61.1%)	

IgA, immunoglobulin A nephropathy; DM, diabetic nephropathy; CGN, chronic glomerulonephritis; others, other diseases including polycystic kidney, focal glomerular sclerosis, nephrosclerosis, lupus nephritis, gestational toxicosis, gouty nephropathy, and other unknown diseases; HD, hemodialysis; PD, peritoneal dialysis.

Table 1B
Patient characteristics after propensity score matching (liver transplantation).

	Incompatible N = 14	Compatible N = 28	P value
Age (years: median, range)	54.5 (20.0–64.0)	55.0 (19.0–63.0)	0.683
BMI	22.85 (16.57–30.42)	21.93 (16.37–33.51)	0.683
Child-Pugh score	10.0 (5.0–13.0)	9.0 (5.0–12.0)	0.722
MELD	15.0 (7.0–20.0)	14.5 (7.0–27.0)	0.864
Gender			1.000
Male	11 (78.6%)	21 (75.0%)	
Female	3 (21.4%)	7 (25.0%)	
Primary disease			0.191
HCV	7 (50.0%)	9 (32.1%)	
HBV	2 (14.3%)	10 (35.7%)	
Alcohol	0 (0%)	3 (10.7%)	
PBC	1 (7.1%)	2 (7.1%)	
NASH	2 (14.3%)	0 (0%)	
HBV + HCV	0 (0%)	0 (0%)	
Others	2 (14.3%)	4 (14.3%)	
A mismatch			1.000
0	3 (21.4%)	6 (21.4%)	
1	8 (57.1%)	17 (60.7%)	
2	3 (21.4%)	5 (17.9%)	
B mismatch			0.718
0	0 (0%)	3 (10.7%)	
1	12 (85.7%)	20 (71.4%)	
2	2 (14.3%)	5 (17.9%)	
DR mismatch			1.000
0	3 (21.4%)	7 (25.0%)	
1	8 (57.1%)	16 (57.1%)	
2	3 (21.4%)	5 (17.9%)	
Splenectomy			0.184
Yes	8 (57.1%)	9 (32.1%)	
No	6 (42.9%)	19 (67.9%)	
Relationship			1.000
Unrelated	4 (28.6%)	9 (32.1%)	
Related	10 (71.4%)	19 (66.7%)	

BMI, body mass index; MELD, model for end stage liver disease score; HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis; NASH, non-alcoholic steatohepatitis; others, other diseases including liver cirrhosis or fulminant hepatitis of uncertain cause, Budd-chiari syndrome, secondary sclerosing cholangitis, liver cirrhosis after surgery of congenital biliary atresia, and liver failure after hepatectomy; Relationship, relationship between recipient and donor.

4. Discussion

Since the first report of prophylactic rituximab administration for ABO-I KT [19], many rituximab protocols for ABO-I KT have been reported [20]. Rituximab has results equivalent to splenectomy, indicating that this invasive surgical procedure is not currently necessary in ABO-I KT. Despite such solid evidence for the beneficial effects of rituximab, the necessary rituximab dose and administration time remain to be elucidated. The dosage of 375 mg/m^2 body surface (lymphoma therapy protocols) has been proven to be safe and efficient [21,22]; hence we employed this dose herein. The effect of lower rituximab doses was also tested on splenic B-cells, and low-dose protocols have been successfully used [23]. To evaluate the clinical merit/demerit of the reduced dose of rituximab treatment, a prospective randomized clinical trial comparing the transplant outcomes and adverse effects of different rituximab doses is necessary. In the published desensitization regimen, the times of rituximab application range from 1 week to 1 month before KT. A further randomized controlled trial is required to better define the optimal timing of rituximab application.

In imitation of the above described desensitization regimen for ABO-I KT, rituximab prophylaxis has been successfully employed in ABO-I adult living-donor LT [6], although the optimal dose and time of rituximab application specific to ABO-I LT also remains to be elucidated. To compare the susceptibility to rituximab of ABO-I KT and LT, we investigated the kinetics of proportions of peripheral blood B-cell subsets in transplant recipients. We observed a slower tempo of B-cell depletion by rituximab in LT recipients than in KT recipients. These findings suggested that administration of rituximab 1 week before transplantation might be inadequate for B-cell depletion in ABO-I LT, whereas it would be adequate in ABO-I KT. One possible reason for a difference in the rapidity of B-cell depletion between KT and LT recipients was different levels of serum complement before desensitization. In patients suffering from liver failure who need LT, complement factors, which are synthesized mainly in the liver, are likely reduced. Since CDC is thought to be an important mechanism for B-cell depletion by rituximab, significantly lower levels of serum complement might contribute to inadequate depletion of B-cells in LT recipients. We also observed a slower tempo of B-cell replenishment in the KT recipients than in LT recipients. It was reported that CD19$^+$ CD5$^+$ B-cell subsets in dialysis patients recovered rapidly, returning to baseline by 6 months after a single dose of rituximab without any other immunosuppression [24]. Another report demonstrated that mycophenolic acid and prednisolone significantly inhibited B-cell proliferation, differentiation, and IgG production [25]. There are two possible mechanisms underlying the differential recovery of KT and LT patients: one is the dose of MMF and steroids (Supplemental Fig. 3), and the other

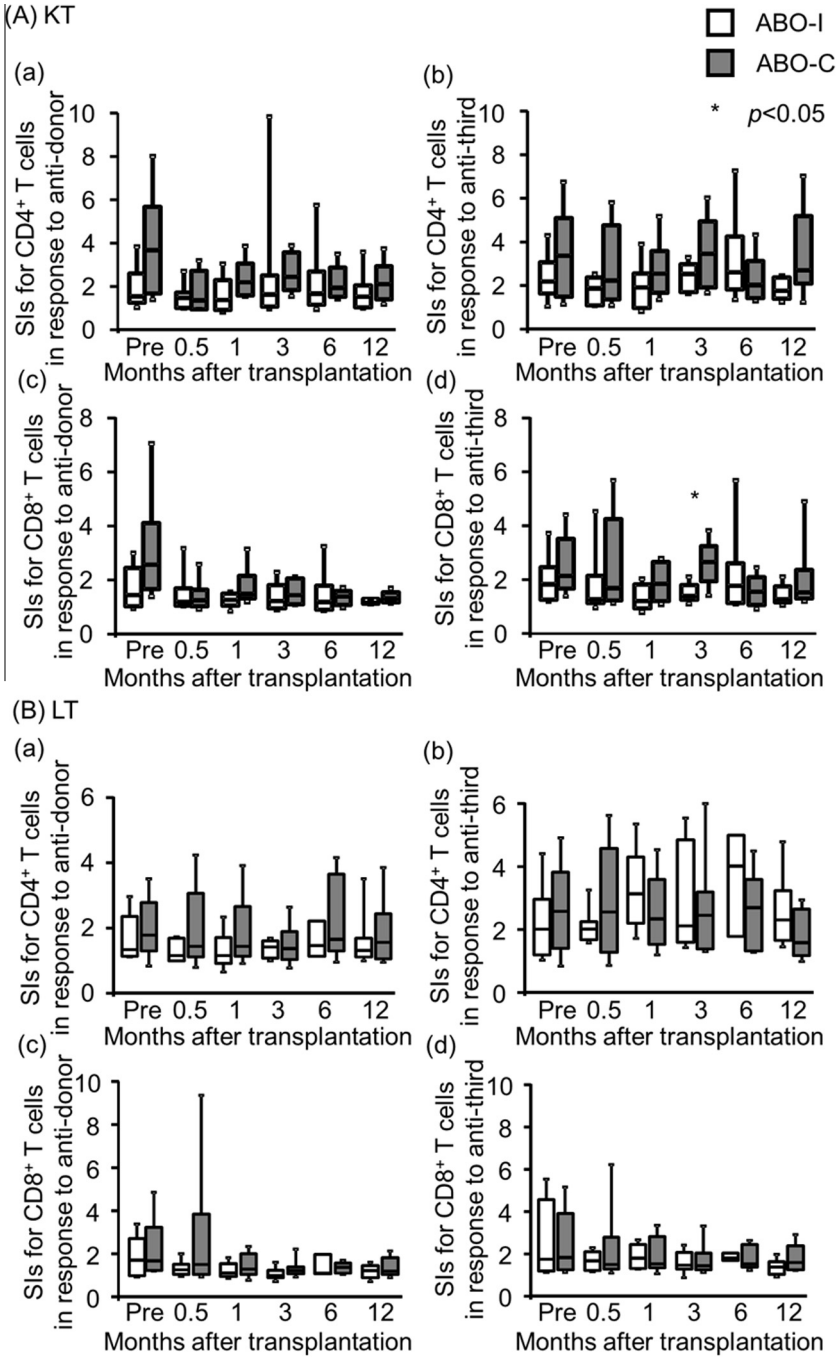

Fig. 6. Kinetics of stimulation index (SI) in the ABO-blood type-incompatible (ABO-I) and ABO-blood type-compatible (ABO-C) groups during the first year after (A) kidney transplantation (KT) and (B) liver transplantation (LT). The SI of each of the $CD4^+$ T-cell (a, b) and $CD8^+$ T-cell (c, d) subsets in the anti-donor (a, c) and anti-third-party (b, d) mixed lymphocyte reactions in patients in the ABO-I group (white box) and ABO-C group (gray box). The box plot indicates the 25th, 50th, and 75th percentiles, and the extended bars represent the 10th through 90th percentiles. The Mann–Whitney *U*-test was used to test differences between ABO-I and ABO-C groups.

is the background of clinical conditions. It has been reported that the capacity of the liver to degrade gut-derived antigens is reduced by the impairment of microcirculation in the cirrhotic liver, resulting in increased release of these antigens into systemic circulation and their redistribution to antibody-forming organs such as the spleen [26]. It has been also reported that cirrhotic patients are likely to be positive for endotoxin. Such clinical conditions might affect the tempo of B-cell recovery.

We investigated the influence of rituximab on T-cells by comparing alloimmune responses between ABO-C and ABO-I KT/LT recipients in MLR assays. Our results indicate that rituximab has minimal effects on the alloreactive T-cell response in both KT and LT recipients. Consistently, in both KT and LT, the incidences of AR and the appearance of dnDSA were not significantly different between the ABO-I and ABO-C groups. One study reported that the percentage of dnDSA production was significantly lower in ABO-I KT recipients treated with rituximab than in ABO-C KT recipients [27], whereas another study reported that the prevalence of dnDSA was not significantly different between ABO-I and ABO-C KT recipients [28]. To clarify these results, a prospective randomized trial that compares the effect of rituximab therapy in ABO-I and ABO-C recipients is needed.

Table 2
Comparison of complications and graft survival between ABO-blood type incompatible (ABO-I) and compatible (ABO-C) groups.

	KT			LT		
	ABO-I (%) N = 18	ABO-C (%) N = 18	P value	ABO-I (%) N = 14	ABO-C (%) N = 28	P value
AR	5.3	10.5	0.50	7.1	10.7	0.59
CMV	52.6	36.8	0.26	71.4	28.6	<0.01
VZV	15.8	15.8	0.67	7.1	10.7	0.59
Fungus	10.5	0.0	0.24	14.3	10.7	0.55
BSI	10.5	15.8	0.50	50.0	28.6	0.15
Neutropenia	31.6	26.3	0.50	21.4	17.9	0.54
De novo DSA 1 year	0	0	–	0	0	–
De novo DSA 2 years	0	0	–	10.0	0	0.34
De novo DSA 3 years	14.3	11.1	0.60	0	0	–
Graft survival 5 years	100	100	–	71.4	79.6	0.43

LT, liver transplantation; KT, kidney transplantation; AR, acute rejection proven by biopsy within 1st year; CMV, cytomegalovirus antigenemia within 1st year; VZV, varicella zoster virus infection within 1st year; Fungus, fungal infection within 1st year; BSI, blood stream infection within 1st year; Neutropenia, neutropenia requiring granulocyte colony stimulating factor within 1st year; *De novo* DSA, HLA-A, B, C, DRB1 or DQB1 antibodies detected against the donor HLA that were not present pre-transplant. MFI values greater than 1000 were considered positive.

In summary, the proportion of the peripheral blood B-cell subset after a single dose of rituximab in KT decreased more rapidly and remained lower than that in LT. In addition, we evaluated the differences of T-cell immunity between ABO-I and ABO-C transplant recipients by MLR assay for the first time to our knowledge, and conclude that rituximab has minimal effects on the alloreactive T-cell responses in KT and LT recipients.

Disclosure

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2016.04.013.

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