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Lower incidence of *de novo* donor-specific antibodies against HLA-DR in ABO-incompatible renal transplantation

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ABSTRACT

Recently, in vitro experiments have demonstrated that anti-blood group A/B antibody binding to endothelial cells induce a protective effect against antibody-mediated injury. This study aimed to clarify the potential clinical benefit of ABO incompatibility in donor-specific HLA antibody (DSA)-induced chronic antibody-mediated rejection (ABMR). We enrolled 215 ABO-incompatible renal transplant (ABO-I) and 467 ABO-identical/compatible renal transplant recipients (ABO-Id/C). The prevalence of *de novo* DSA production and incidence of biopsy-proven chronic ABMR were compared between the two groups. The incidence of DR-associated *de novo* DSA was significantly lower in ABO-I than in ABO-Id/C ($P = 0.028$). Diagnostic biopsy for ABMR was conducted in 54 patients (11 ABO-I and 43 ABO-Id/C). Biopsy-proven chronic ABMR was lower in ABO-I than in ABO-Id/C (27.3% [3/11] vs. 44.2% [19/43]) patients. Our findings suggest that ABO incompatibility may cause low production of DR-associated *de novo* DSA, possibly resulting in a reduced incidence of chronic ABMR.

1. Introduction

The presence of donor-specific HLA antibody (DSA) remains a complicated problem that causes antibody-mediated rejection (ABMR), followed by poor graft survival [1–3]. To date, effective treatment of or prevention against chronic ABMR has not been established. However, the outcome of ABO-incompatible renal transplantation (ABO-I) is comparable to that of ABO-identical or compatible renal transplantation (ABO-Id/C) [4–9]. In ABO-I patients, accommodation, a phenomenon in which the transplanted organ is not rejected even in the presence of anti-donor antibodies, has been clinically observed and recognized as an important factor for graft survival [10–12]. Although the mechanism of accommodation is still unclear, it has been previously demonstrated that anti-blood group A/B antibody (A/B antibody) ligation-induced resistance to HLA antibody-mediated, complement-dependent cytotoxicity (CDC) through the upregulation of complement regulatory proteins and downregulation of HLA-DR expression in vitro [13–15]; however, whether this protective effect against CDC can be observed in ABO-I is yet to be verified. In this study, we investigated the clinical impact of ABO incompatibility on the development of *de novo* DSA and chronic ABMR.

2. Material and methods

2.1. Study subjects

This study is a retrospective observational cohort study of 781 consecutive subjects who received living donor renal transplantation

Abbreviations: A/B antibody, anti-blood group A/B antibody; ABO-I, ABO-incompatible renal transplantation; ABO-I w/o R/S, ABO-incompatible renal transplantation treated with neither rituximab nor splenectomy; ABO-I with R/S, ABO-incompatible renal transplantation treated with rituximab or splenectomy; ABO-Id/C, ABO-identical/compatible renal transplantation; ABMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; DSA, donor-specific HLA antibody; MFI, mean fluorescence intensity; TCMR, T cell-mediated rejection

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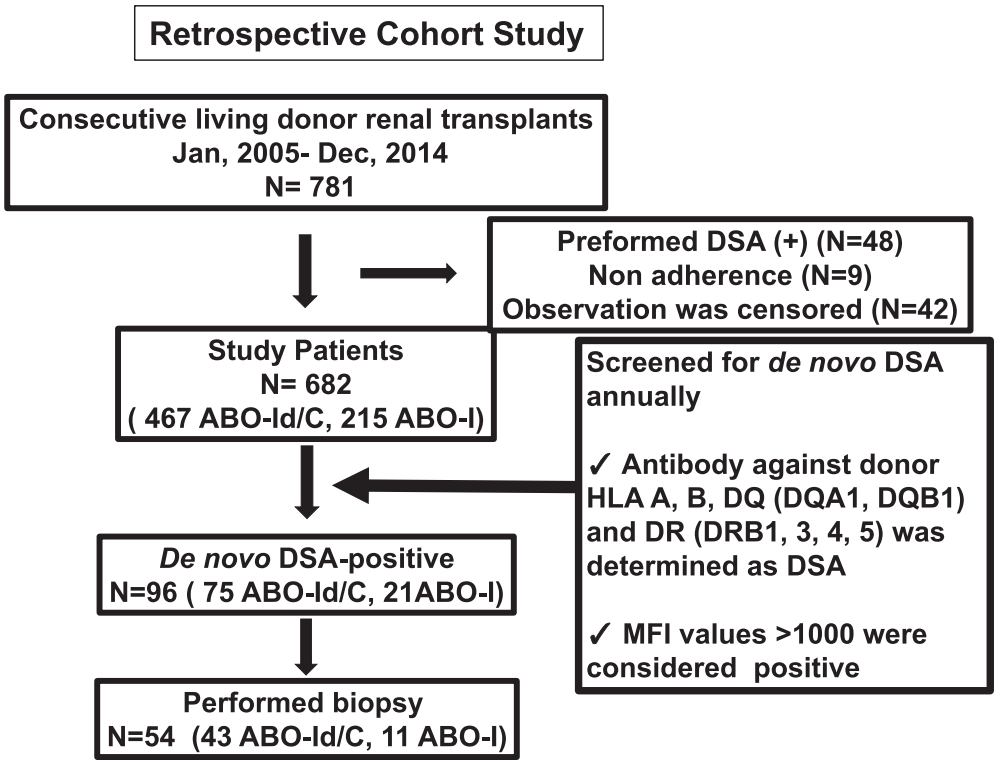

Fig. 1. Retrospective cohort study of 682 consecutive renal transplantations. Seven hundred and eighty-one living donor renal transplantations were performed between 2005 and 2014. After the exclusion of 90 patients who had pretransplant DSA or could not be followed up, and 9 non-adherent patients, 682 patients were included in this retrospective cohort study. Annual examination of *de novo* DSA was performed. Among 682 recipients, 96 developed *de novo* DSA, of which 54 consented to receive diagnostic biopsy for chronic ABMR. (ABO-Id/C, ABO-identical/compatible renal transplantation; ABO-I, ABO-incompatible renal transplantation; DSA, donor-specific HLA antibody; MFI, mean fluorescence intensity).

Table 1
Patients' background in ABO-I and ABO-Id/C group.

	ABO-I (N = 215)	ABO-Id/C (N = 467)	*P* value
Follow up period (month)	66 (47, 95)	76 (52, 105)	0.005
Age (years)	51 (38, 60)	40 (31, 54)	< 0.001
Male/Female	148/67	293/174	0.143
Relationship (%)			
Parent and child	70 (32.6)	252 (54.0)	< 0.001
Sibling	14 (6.4)	45 (9.6)	
Spouse	126 (58.6)	157 (33.6)	
Others	5 (2.3)	13 (2.8)	
Number of HLA-A, B mismatch (%)			< 0.001
0	12 (5.6)	28 (6.0)	
1	31 (14.2)	88 (18.8)	
2	77 (35.8)	234 (50.1)	
3	59 (27.4)	83 (17.8)	
4	36 (16.7)	34 (7.3)	
Number of HLA-DRB1 mismatch (%)			< 0.001
0	18 (8.4)	48 (10.3)	
1	107 (49.8)	297 (63.6)	
2	90 (41.9)	122 (26.1)	
Cyclosporine/Tacrolimus (%)	137 (63.7)/78 (36.3)	254 (54.4)/213 (45.6)	0.025
Trough of cyclosporine (ng/ml)	95.9 (81.3, 111.1)	95.7 (79.4, 116.2)	0.662
Trough of tacrolimus (ng/ml)	5.72 (4.90, 6.47)	5.44 (4.71, 6.06)	0.189
Rituximab (%)	119 (55.3)	1 (0.2)	< 0.001
Splenectomy (%)	44 (20.5)	1 (0.2)	< 0.001

Parenthesis of continuous variables include Interquartile ranges. Abbreviations: ABO-I, ABO incompatible living donor renal transplantation; ABO-Id/C, ABO identical or compatible living donor renal transplantation.

between 2005 and 2014 at Nagoya Daini Red Cross Hospital. Among these 781 recipients (Fig. 1), those who had pretransplant DSA (n = 48) and who could not be followed up due to transfer of care to a remote center (n = 42) were excluded. Nine patients were also excluded because of obvious nonadherence. A total of 682 maintenance recipients were included in this study. The median post-transplantation follow-up period was 6.2 years (range, 0.8–11.8 years). Maintenance immunosuppressive therapy consisted of a calcineurin inhibitor (cyclosporine or tacrolimus), steroid (prednisolone or methylprednisolone), and antimetabolites (mycophenolate mofetil or mizoribine) or mTOR inhibitor (everolimus). All the patients received basiliximab intravenously as induction therapy. Recipients of ABO-I were additionally pretreated with mycophenolate mofetil from day −14 as well as double-filtration plasmapheresis and either splenectomy, rituximab (200 mg/body; two times; days −14 and −1), or neither (due to low A/B antibody titers).

2.2. Study design

Among 682 consecutive recipients, 467 and 215 patients who received ABO-Id/C and ABO-I, respectively, were annually examined for incidence, specificity, number, and mean fluorescence intensity (MFI) of *de novo* DSA since 2006. The outcomes of *de novo* DSA and rejection during the study period were compared between the ABO-Id/C and ABO-I groups. In addition, among recipients who developed *de novo* DSA (n = 96), those who provided consent (n = 54) received a renal biopsy to diagnose chronic ABMR and were divided into chronic ABMR and no ABMR groups. The characteristics of *de novo* DSA were also compared between the two groups. The end date for the analysis was December 31, 2016.

Written informed consent was obtained from all patients. This study was approved by the institutional review board of Nagoya Daini Red Cross Hospital and by the institutional ethics committee of Aichi Medical University School of Medicine, in accordance with the current standards for human research as outlined in the Declaration of Helsinki.

2.3. DSA detection and identification

Serum samples collected annually from 2006 to 2016 were examined for IgG antibodies against HLA class I or II. Enzyme-linked

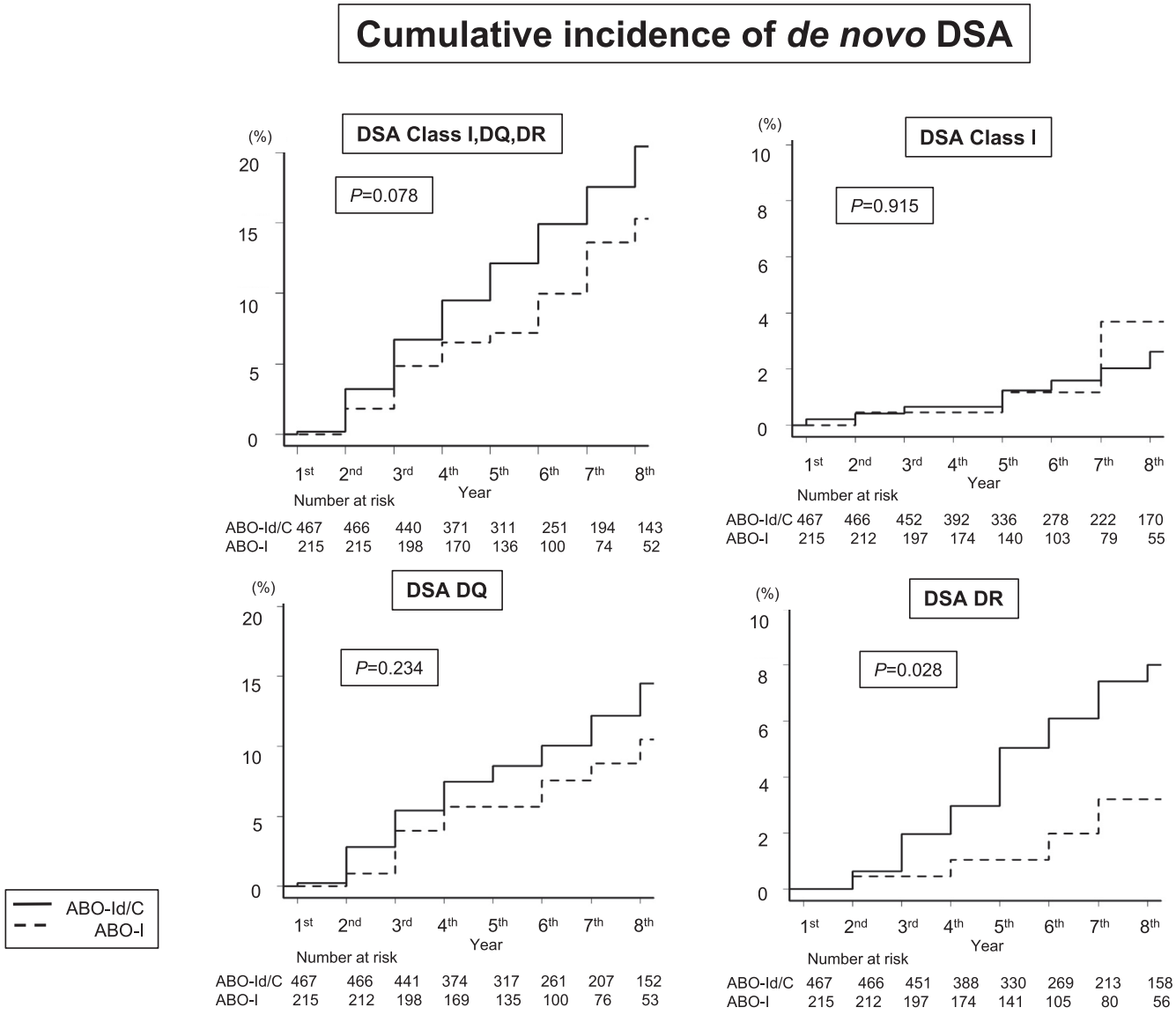

Fig. 2. Cumulative incidence of *de novo* DSA in ABO-I and ABO-Id/C patients. The incidence of DSAs against DR was significantly lower in ABO-I patients than in ABO-Id/C patients ($P = 0.028$), whereas the incidence of DSAs against class I or DQ did not differ significantly between the two groups ($P = 0.915$ and $P = 0.234$, respectively). Cumulative incidence was tested using the Gray-test. (ABO-Id/C, ABO-identical/compatible renal transplantation; ABO-I, ABO-incompatible renal transplantation; DSA, donor-specific HLA antibody).

immunosorbent assay was used between 2006 and 2007, and Flow PRA or LABScreen Mixed kits (One Lambda, Canoga Park, CA) were employed for the screening since 2008. After screening, DSA was identified in HLA antibody-positive recipients using LABScreen single antigen beads (One Lambda). DSAs against A, B, DR (DRB1,-3,-4,-5), and DQ (DQA1, DQB1) were determined, and MFI values > 1000 were considered positive. To distinguish between preformed and *de novo* antibodies, sera conserved before transplantation were also examined for HLA antibody.

2.4. Diagnosis of rejection

Renal biopsy was performed only after the consent of the recipients who developed *de novo* DSA. Biopsy specimens were analyzed using light microscopy, electron microscopy, and immunofluorescence methods. ABMR, and T cell-mediated rejection (TCMR) were diagnosed according to the 2013 Banff classification of renal allografts.

2.5. Statistical analyses

Nominal variables were examined using Fisher's exact test. Continuous variables and ordinal variables were tested using the Mann–Whitney *U* test. The cumulative incidence of *de novo* DSA was tested using the Gray-test. *P* values of < 0.05 were considered statistically significant.

3. Results

3.1. Patient backgrounds

Compared with ABO-Id/C patients, ABO-I patients had a significantly shorter follow-up time, higher age, higher rate of HLA-A, B and DRB1 mismatch, and higher proportion of cyclosporine use (Table 1). Approximately 70% of ABO-I patients underwent splenectomy or rituximab desensitization treatment in addition to basiliximab induction therapy, whereas ABO-Id/C patients received only basiliximab induction therapy, except for one patient who was treated

Table 2
Outcomes of *de novo* DSA and rejection in ABO-I and ABO-Id/C group.

	ABO-I (N = 215)	ABO-Id/C (N = 467)	*P* value
De novo DSA			
Class I-associated (%)	4 (1.9)	10 (2.1)	1
DQ-associated (%)	16 (7.4)	53 (11.3)	0.133
DR-associated (%)	4 (1.9)	29 (6.2)	0.024
Class I alone (%)	2 (0.9)	6 (1.3)	1
Class I + DQ (%)	1 (0.5)	3 (0.6)	1
DQ alone (%)	14 (6.5)	37 (7.9)	0.639
DR alone (%)	2 (0.9)	16 (3.4)	0.072
DQ + DR (%)	1 (0.5)	12 (2.7)	0.073
Class I + DR (%)	1 (0.5)	0	0.315
Class I + DQ + DR (%)	0	1 (0.2)	1
Acute TCMR (%)	29 (13.5)	56 (10.9)	0.37
ABMR within 2 weeks after transplantation (%)	6 (2.8)	0 (0.0)	0.001
Chronic ABMR (diagnostic biopsy) (%)	3/11 (27.3)	19/43 (44.2)	0.493
MFI of *de novo* DSA (IQR)	4907 (1257, 11386)	6719 (2890, 17649)	0.481

Abbreviations: ABMR, antibody-mediated rejection; ABO-I, ABO incompatible living donor renal transplantation; ABO-Id/C, ABO identical or compatible living donor renal transplantation; DSA, donor-specific HLA antibody; IQR, interquartile range; MFI, mean fluorescence intensity; TCMR, T cell-mediated rejection.

with rituximab for prevention of the recurrence of focal segmental glomerulosclerosis, and another who received splenectomy for the treatment of thrombotic thrombocytopenic purpura. No significant differences were found in gender or trough levels of calcineurin inhibitors between the two groups.

3.2. De novo DSA

Ninety six out of 682 patients developed *de novo* DSA during the study period. The cumulative incidence of *de novo* DSAs as a whole didn't significantly differ between ABO-I and ABO-Id/C ($P = 0.078$, Fig. 2). However, regarding the specificity of *de novo* DSA, the cumulative incidence of DR-associated DSA was significantly lower in ABO-I patients than in ABO-Id/C patients ($P = 0.028$), whereas the incidence of DSAs against class I or DQ did not differ between the two groups ($P = 0.915$ and $P = 0.234$, respectively, Fig. 2). There was no significant difference in total MFI of *de novo* DSAs between the ABO-I and ABO-Id/C groups (Table 2).

On multivariate analysis using Cox proportional hazard regression model, ABO-incompatibility seemed to be associated with low incidence of DR-associated *de novo* DSA, although it did not slightly reach statistical difference (hazard ratio 0.348, 95% confidence interval 0.121–1.001, $P = 0.050$, Table S1). By contrast, no significant difference in the cumulative incidence of *de novo* DSA was observed between the ABO-I with and without rituximab/splenectomy pretreatment groups (Fig. 3). In addition, both trough levels of cyclosporine and tacrolimus didn't significantly differ depending on with or without *de novo* DSA development, either (Table S2).

3.3. Rejection

The incidence of TCMR did not differ between the ABO-I and ABO-Id/C groups. On the other hand, the incidence of ABMR within 2 weeks after transplantation was significantly higher in ABO-I patients than in ABO-Id/C patients (2.8% [6/215] vs. 0.0% [0/467], $P = 0.001$, Table 2). In all the cases of ABMR that occurred within 2 weeks after transplantation, DSA was not detected in the sera. Therefore, these ABMRs were considered to be caused by not DSA but A/B antibody. These ABMR cases were treated by steroid pulse therapy and plasma exchange with/without rituximab treatment and recovered their renal function (data not shown). Among 96 patients with *de novo* DSA, 54 patients underwent diagnostic biopsy. Although estimated GFR didn't significantly differ between the 54 patients and remaining 42 patients (39.9 vs 45.1 mL/min/1.73 m^2, $P = 0.188$), overt proteinuria (> 0.5 g/day) was observed in 10 out of 54 patients at the time of diagnostic biopsy. The incidence of biopsy-proven chronic ABMR was lower in ABO-I patients than in ABO-Id/C patients, but this difference was not significant (27.3% [3/11] vs. 44.2% [19/43], $P = 0.493$, Table 2). In recipients who received diagnostic biopsy, the proportions of DR-associated DSA was significantly higher in patients with biopsy-proven chronic ABMR than in those with no biopsy findings of chronic ABMR (54.5% [12/22] vs. 15.6% [5/32], $P = 0.003$, Table 3). The number and MFI of *de novo* DSAs were also higher in patients with chronic ABMR than in those with biopsy-negative chronic ABMR ($P = 0.003$ and 0.048, respectively, Table 3). Pathological findings of 54 patients who underwent diagnostic biopsy are shown in Table 4. Microvascular inflammation (g + ptc) and transplant glomerulopathy (cg) scores tended to be lower in the ABO-I than in ABO-Id/C cases. Among ABO-I patients, the incidence of acute TCMR and ABMR did not differ according to the presence or absence of rituximab/splenectomy pretreatment (Table 5).

4. Discussion

The mechanism of accommodation is yet to be clarified. Many researchers have investigated antibody ligation-induced immune response, intracellular signaling pathways, and gene regulation [16–18]. Iwasaki et al. reported a protective effect induced by A/B antibody. We demonstrated that A/B antibodies binding to the endothelium induced resistance to HLA-DR antibody-mediated CDC, which might be caused by the downregulation of HLA-DR expression through inactivation of mTOR pathway [13] in addition to upregulation of complement regulatory proteins (CD55 and CD59) through inactivation of ERK pathway [14]. This protective effect may elicit favorable medium- and long-term graft outcomes in ABO-I despite the increased incidence of acute ABMR. However, the clinical benefit of ABO incompatibility in the development of *de novo* DSA and chronic ABMR has not yet been investigated.

In the current study, we compared the incidence of *de novo* DSA and chronic ABMR between ABO-I and ABO-Id/C patients. Interestingly, the incidence of *de novo* DR-associated DSA was significantly lower in ABO-I patients than in ABO-Id/C patients, whereas there was no significant difference in the incidence of DSAs against class I or DQ. Furthermore, ABO-I patients had a considerably lower incidence of chronic ABMR than did ABO-Id/C patients, although the difference did not reach statistical significance. These clinical findings correspond to the results of the in vitro study reported by Iwasaki et al [13]. In addition, renal dysfunction due to chronic ABMR is reported to be strongly associated with DSAs against class II. Although DQ-associated DSA is most frequently detected, the coexistence of both DR- and DQ-associated DSAs seems to be closely related to chronic ABMR compared with the presence of DQ-associated DSA alone [19–22]. Mechanisms of immune response toward HLA antibody production may differ among DQ DSA and other DSAs. Given that (i) compared with other DSAs, a high prevalence of DQ-associated DSA was observed, but the presence of DQ DSA alone did not always cause chronic ABMR [19–21], as well as that (ii) graft endothelial cells under static conditions expressed HLA-DQ at a very low or negligible level unlike DR [23,24], the triggering of DQ DSA production may not be attributable to graft endothelial cells. By contrast, the production of DR DSA may be related to graft injury. Thus, co-existent DQ DSA could promote ABMR once HLA-DQ expression is upregulated in activated graft endothelial cells. The lower incidence of DR-associated DSA in ABO-I patients may possibly contribute to long-term graft survival. When accommodation is established, endothelial

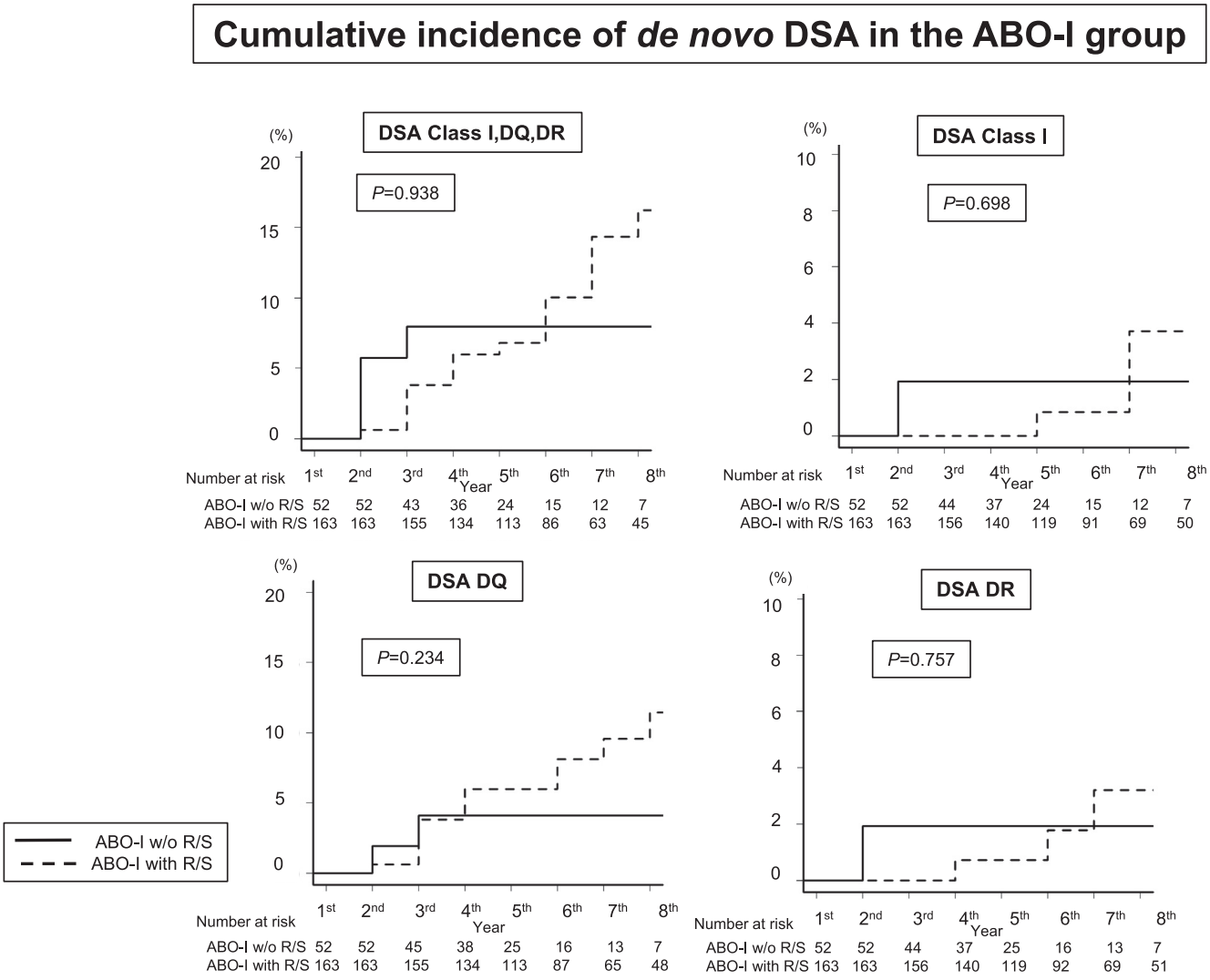

Fig. 3. Cumulative incidence of *de novo* DSA in ABO-I patients. There were no significant differences in the incidence of DSAs against class I, DQ and DR between the ABO-I group treated with rituximab or splenectomy and the ABO-I group with neither rituximab nor splenectomy ($P = 0.698$, $P = 0.234$ and $P = 0.757$ respectively). Cumulative incidence was tested using the Gray-test. (ABO-I w/o R/S, ABO-incompatible renal transplantation treated with neither rituximab nor splenectomy; ABO-I with R/S, ABO-incompatible renal transplantation treated with rituximab or splenectomy; DSA, donor-specific HLA antibody).

cells can exhibit good resistance to graft injury caused by not only anti-A/B antibody, but also HLA antibody including DR, DQ or class I DSA. This study could indicate the protective effect of ABO-I on only DR-associated DSA, probably due to low levels of DQ expression in endothelial cells and prevalence of de novo class I DSA. Analysis in a large number of cases may clarify the significant effect of DQ and class I DSA.

The outcome of ABO-I patients with pretransplant DSA has also been reported to be worse than that of ABO-I patients [25]. Considering that DSA-dependent, complement, or thrombin-induced endothelial activation immediately after transplantation could inhibit the acquisition of protective effect [14], co-existing anti-A/B antibodies may even accelerate antibody-mediated graft injury. A critical period without graft injury of 1–2 weeks after transplantation is necessary for the induction and establishment of graft accommodation in ABO-I patients [26]. Therefore, clinical benefit from ABO incompatibility would not be expected in patients with pretransplant DSA, unlike *de novo* DSA. The medium-term effect of rituximab on the production of *de novo* DSA may be controversial [4,27]. We have reported that the development of *de novo* HLA antibody was hardly influenced by pretreatment with rituximab or splenectomy during medium-term follow-up, because the incidence of *de novo* HLA antibody was similar between ABO-Id/C and ABO-I [27]. Likewise, cumulative incidence of *de novo* DSA as a whole was not significantly different between the two groups in the current study. However, the incidence of *de novo* DR-associated DSA was lower in ABO-I than in ABO-Id/C. In addition, there was no difference in the incidence of *de novo* DSA and chronic ABMR according to the presence or absence of rituximab treatment. Furthermore, it is equally unlikely that mycophenolate mofetil pretreatment starting two weeks before transplantation would influence de novo DSA production during medium-term follow up. Hence, we consider that accommodation by A/B antibody is one possible explanation for the lower incidence of DR-associated DSA in ABO-I patients than in ABO-Id/C patients.

The major limitation of this study is that the number of diagnostic biopsy was very small, because of difficulty in obtaining informed consent from de novo DSA-positive patients in the maintenance period. Other limitations include a retrospective single-center design, lack of long-term data of A/B antibody titers, and heterogeneity of patients' background and immunosuppression protocols in each group. Further analysis in larger studies with longer follow-up periods is required to provide confirmatory evidence, particularly regarding graft outcomes.

In conclusion, a lower incidence of *de novo* DR-associated DSA was observed in ABO-I patients, who may be less vulnerable to *de novo* DSA-

Table 3
Outcomes of *de novo* DSA in the cases who received diagnostic biopsy for chronic ABMR.

	No chronic ABMR (n = 32)	Chronic ABMR (n = 22)	*P* value
Period between transplantation and biopsy (month)(IQR)	56.0 (34.8, 69.0)	54.5 (38.5, 70.5)	0.833
De novo DSA			
Class I-associated (%)	8 (25.0)	2 (9.1)	0.173
DQ-associated (%)	23 (71.9)	20 (90.9)	0.167
DR-associated (%)	5 (15.6)	12 (54.5)	0.003
Class I alone (%)	6 (18.2)	0 (0)	0.071
Class I + DQ (%)	2 (6.1)	1 (4.5)	1
DQ alone (%)	19 (60.6)	9 (40.9)	0.268
DR alone (%)	3 (9.1)	2 (9.1)	1
DQ + DR (%)	2 (6.1)	9 (40.9)	0.004
Class I + DQ + DR (%)	0 (0)	1 (4.5)	0.407
Number of DSA (%)			0.003
1	27 (84.4)	10 (45.5)	
2	4 (12.5)	10 (45.5)	
3	0 (0.0)	2 (9.1)	
4	1 (3.1)	0 (0.0)	
MFI of *de novo* DSA (IQR)	5951 (1655, 11075)	10,952 (4820, 22355)	0.048

Abbreviations: DSA, donor-specific HLA antibody; ABMR, antibody-mediated rejection; IQR, interquartile range.

Table 4
Pathological findings of diagnostic biopsy in the cases with *de novo* DSA.

	ABO-I (N = 11)	ABO-Id/C (N = 43)	*P* value
Chronic ABMR	3/11	19/43	0.493
g + ptc score(%)			0.516
0	7 (63.6)	18 (41.9)	
1–3	3 (27.3)	19 (44.2)	
4–6	1 (9.1)	6 (14.0)	
cg score(%)			0.499
0	7 (63.6)	21 (48.8)	
1	3 (27.3)	9 (20.9)	
2	0 (0.0)	2 (18.6)	
3	1 (9.1)	5 (11.6)	
C4d positive in ptc/cg(%)	4 (36.4)	16 (37.2)	1
Type of DSA			0.575
Class I	2 (18.2)	4 (9.3)	
DQ	7 (63.6)	21 (48.8)	
DR	0 (0.0)	5 (11.6)	
Class I + DQ	1 (9.1)	2 (4.7)	
DQ + DR	1 (9.1)	10 (23.3)	
Class I + DQ + DR	0 (0.0)	1 (2.3)	
Total MFI of DSA (IQR)	4907 (1466, 10681)	6731 (3090, 19010)	0.308

Abbreviations: ABMR, antibody-mediated rejection; ABO-I, ABO incompatible living donor renal transplantation; ABO-Id/C, ABO identical or compatible living donor renal transplantation; DSA; donor-specific HLA antibody; IQR, interquartile range; MFI, mean fluorescence intensity.

mediated chronic ABMR. It is suggested that graft accommodation in ABO-I patients might exhibit a protective effect against not only anti-A/B antibody- but also *de novo* DSA-induced graft injury.

Authors' contributions

M.O., Y.W. and T.K. designed the research; M.O. and T.K. wrote the paper; M.O., K.I., K.F., T.Y., T.H., M.T., N.G., S.N., A.T. and T.K. performed the research; M.O. and T.K. participated in data analysis.

Table 5
Outcomes of *de novo* DSA and rejection in ABO-I group with and without rituximab or splenectomy treatment.

	ABO-I without rituximab or splenectomy (n = 52)	ABO-I with rituximab or splenectomy (n = 163)	*P* value
Median (IQR) titer of A/B IgG	2 (2, 8)	32 (4128)	< 0.001
De novo DSA (%)			
Class I-associated (%)	1 (1.9)	3 (1.8)	1
DQ-associated (%)	2 (3.8)	14 (8.6)	0.513
DR-associated (%)	1 (1.9)	3 (1.8)	1
Class I alone (%)	1 (1.9)	1 (0.6)	0.426
Class I + DQ (%)	0	1 (0.6)	1
DQ alone (%)	2 (3.8)	12 (7.4)	0.526
DR alone (%)	1 (1.9)	1 (0.6)	0.426
DQ + DR (%)	0	1 (0.6)	1
Class I + DR (%)	0	1 (0.6)	1
Number of DSA (%)			1
1	4 (100.0)	13 (75.0)	
2	0 (0.0)	3 (20.0)	
3	0 (0.0)	1 (5.0)	
Acute TCMR (%)	8 (15.4)	21 (12.9)	0.645
ABMR within 2 weeks after transplantation (%)	2 (3.8)	4 (2.5)	0.633
Chronic ABMR (%)	0 (0.0)	3 (1.8)	1

Abbreviations: DSA, donor-specific HLA antibody; ABO-I, ABO incompatible living donor renal transplantation; TCMR, T cell-mediated rejection; ABMR, antibody-mediated rejection; IQR, interquartile range.

Declarations of interest

None.

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Appendix A. Supplementary data

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