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Low Titers of Antidonor ABO Antibodies After ABO-Incompatible Living Donor Liver Transplantation: A Long-Term Follow-Up Study

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Background. The ABO blood-type barrier in kidney and liver transplantation has been overcome by aggressive treatments such as B cell depletion using rituximab. However, the long-term effects of ABO-incompatible liver transplantation (ABO-I LTx) on immunological status have not previously been studied. Here, we assessed whether long-term immune hyporesponsiveness against ABO blood-group antigens was retained. **Methods.** We recruited 81 patients, 75 patients who had survived ABO-I LTx without retransplantation and 6 patients who had survived after retransplantation using blood type-compatible grafts. The time between ABO-I LTx and outpatient visits for blood sampling for this study ranged from 1.1 to 16.8 years. We also evaluated patients' backgrounds and postoperative therapies. **Results.** Overall, antidonor ABO antibody titers in the 75 patients without retransplantation decreased during long-term follow-up. In the subset of 40 patients with blood type O, anti-nondonor ABO antibody titers did not decrease and were significantly higher than antidonor ABO antibody titers. In addition, long-term antidonor ABO antibody titers were significantly lower in pediatric patients than in adult patients. In the 6 patients who were retransplanted with blood type-compatible grafts, antidonor ABO antibody immunoglobulin G titers remained low, but IgM titers increased slightly long after removal of the ABO-incompatible graft. **Conclusions.** These findings suggest that donor-specific hyporesponsiveness remains after ABO-I LTx, particularly in pediatric patients. Long-term persistence of blood antigens may contribute to this donor-specific hyporesponsiveness.

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The prognosis in ABO-incompatible liver transplantation (ABO-I LTx) is inferior to that in ABO-identical/-compatible LTx.^{1,2} However, living donor liver transplantation of ABO-I grafts is performed in Japan because of the shortage of deceased donors.

Complications associated with antibody-mediated rejection (AMR) due to the presence of antidonor ABO antibodies decrease the favorable nature of the prognosis after ABO-I LTx. Antidonor ABO antibody titers often increase during the perioperative period, even when preoperative desensitization therapy has been performed.^{3,4} However, the prognosis in ABO-I LTx is similar to that in ABO-identical/-compatible LTx when AMR does not occur in the perioperative period.^{1,5} AMR may not occur until several years after not only LTx but also heart transplantation⁶ and kidney transplantation.⁷

In a long-term follow-up study, to clarify whether the long-term stability of graft function is due to immune hyporesponsiveness or to accommodation against ABO blood-group antigens, we assessed the antidonor ABO antibody titers of patients who had undergone ABO-I LTx.

MATERIALS AND METHODS

Patient Characteristics

In our hospital, we performed ABO-I LTx on 250 patients from 1987 through 2011; 91 of these patients died, 16 were

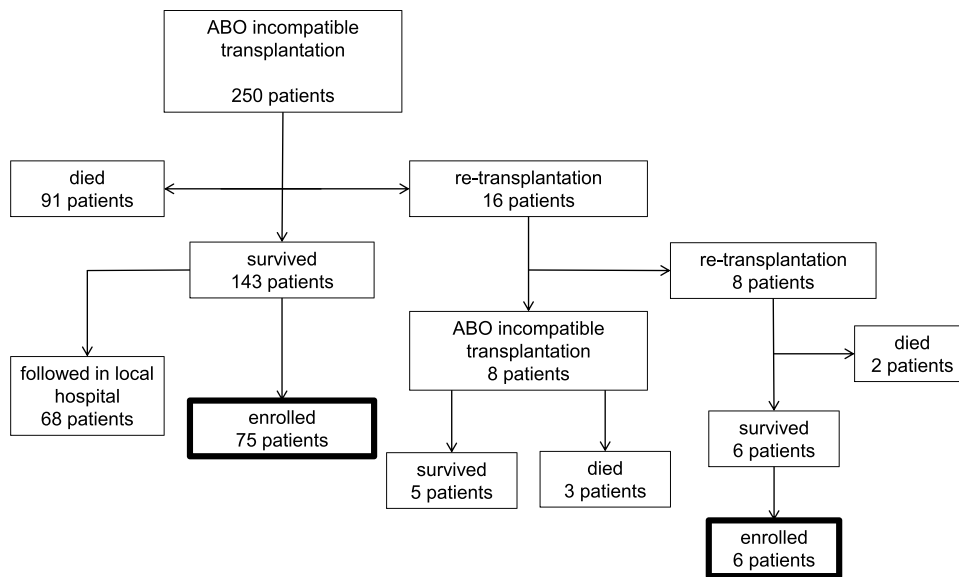


FIGURE 1. Flowchart of patient enrollment. In our hospital, we performed ABO-incompatible liver transplantation (ABO-I LTx) on 250 patients from 1987 to 2011; of these, 91 patients died, 16 were retransplanted, and 143 survived without retransplantation. Among the 16 retransplanted patients, 8 underwent a second ABO-I LTx, and the remaining 8 received ABO-identical/-compatible LTx at retransplantation; 6 of these latter 8 patients survived. We enrolled 75 of the 143 patients who survived without retransplantation and the 6 patients who received ABO-identical/-compatible LTx at retransplantation into the current study.

retransplanted, and 143 survived without retransplantation until December 2012. Among the 16 retransplanted patients, 8 again received ABO-I LTx, whereas the remaining 8 underwent ABO-identical/-compatible LTx; 6 of the latter group survived. To measure the antibody titers of patients who survived long-term after LTx, we asked them to visit the outpatient clinic for blood sampling; we enrolled 75 of the 143 patients who survived ABO-I LTx without retransplantation and the 6 patients who received ABO-identical/-compatible LTx for retransplantation (Figure 1). We excluded 68 patients who survived without retransplantation with stable condition living far away from our hospital with followed-up by their local hospital. We also excluded the 8 patients who underwent retransplantation with ABO-I LTx because of the difficulty in evaluating the influences of the 2 ABO-I LTx procedures on antidonor ABO antibody titers. All recipients underwent LTx by standard methods.⁸

Immunosuppression and Desensitization Therapy

In patients who underwent ABO-I LTx, desensitization therapy was performed as previously described,^{9,10} including plasma exchange or exchange transfusion, local infusion (portal or arterial), and rituximab administration.

In addition, all patients who underwent ABO-I LTx received preoperative plasmapheresis or blood exchange to reduce the antidonor ABO antibody titer to less than 16. Beginning in 2004, rituximab was administered preoperatively at a dose of 300 mg for patients older than 15 years or 375 mg/m² for 1- to 15-year-olds; rituximab was not used in patients younger than 1 year.

From 2000 to 2010, local infusion therapy was performed postoperatively as previously described in patients older than 2 years who underwent ABO-I LTx, to control local disseminated intravascular coagulation in grafts.¹¹ Briefly, prostaglandin E1 (0.01 µg/kg per minute for 3 weeks) and

methylprednisolone (125 mg daily for the first 7 days and 50 mg daily for another 7 days) were administered through a catheter inserted into the portal vein or hepatic artery (or both) during surgery.^{12,13} The numbers of patients treated with local infusion therapy are shown in Table 2.

Splenectomy was performed on all adult patients who underwent ABO-I LTx until 2004. From 2004 onward, splenectomy was performed only in recipients with hepatitis C virus infection (to prevent a decrease in the platelet count during anti-hepatitis C virus therapy) or in those who received a small-for-size graft (to modulate the portal pressure).¹⁴

Drugs used for induction of immunosuppression in ABO-I LTx consisted mainly of tacrolimus, steroids, and mycophenolate mofetil. The serum level of tacrolimus was 10–15 ng/mL during the first 2 weeks, 10 ng/mL during the next month, and 5–8 ng/mL during the following 6 months. Patients diagnosed with acute cellular rejection were treated with steroid pulse therapy.

Diagnosis of Rejection and Treatment

Patients whose antidonor ABO antibody titers exceeded 128 and in whom clinical AMR was suspected underwent plasma exchange at the judgment of the attending physicians. Acute cellular rejection was diagnosed by liver biopsy to Banff criteria. Also, chronic rejection was diagnosed by histopathology according to Banff criteria.^{15,16} ABO-I-related AMR was diagnosed in patients with elevated antidonor ABO antibody titers, graft dysfunction, and liver biopsy findings compatible with AMR, including C4d deposition and periportal edema and necrosis.¹⁷ Patients with ABO-I-related AMR were treated with steroid pulse therapy, high-dose intravenous immunoglobulins, and plasmapheresis.³ Anti-HLA donor-specific antibody titers during AMR were not measured in this study.

TABLE 1.
Background of 75 patients surviving without retransplantation

Patient demographic characteristics	
No. patients	75
Age at LTx, y	38.7 (0.08–66.73)
Years after LTx	6.2 (1.1–16.8)
Sex (female)	44 (58.7%)
Original disease	
Biliary atresia	30
Acute liver failure	3
HCV	14
HBV	12
Primary biliary cirrhosis	7
Alcoholic liver cirrhosis	2
Autoimmune hepatitis	1
Nonalcoholic steatohepatitis	1
Postoperative bile duct stenosis	1
Primary sclerosing cholangitis	1
Progressive familial intrahepatic cholestasis	1
Liver cirrhosis (non-B, non-C)	1
Congenital hepatic fibrosis	1
Perioperative background	
Preoperative administration of rituximab	39 (52.7%)
Graft-to-recipient weight ratio (%)	1.2 (0.6–4.6)
Splenectomy	27 (36.0%)
Donor-to-recipient blood type	
A to O	25
A to B	8
B to O	13
B to A	8
AB to O	2
AB to A	9
AB to B	10
Crossmatch positive	0
Rejection episodes	
ACR	39 (52.0%)
AMR	7 (9.3%)
Laboratory data at outpatient visit for final titer examination	
Aspartate transaminase, IU/L	27 (14–96)
Alanine transaminase, IU/L	17 (7–137)
Gamma-glutamyltransferase, IU/L	26 (8–538)
Total bilirubin, mg/dL	0.5 (0.1–3.7)
Bile acid, mmol/L	13 (2–209)
Immunosuppression at outpatient visit for final titer examination	
CNI (tacrolimus/cyclosporin) only	37 (49.3%)
CNI + MMF	21 (28.0%)
CNI + PSL	4 (5.3%)
CNI + MMF + PSL	9 (12.0%)
CNI + MIZ + PSL	1 (1.3%)
CNI + rapamycin	2 (2.7%)
MMF without CNI	1 (1.3%)

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CNI, calcineurin inhibitor; HBV, hepatitis B virus; HCV, hepatitis C virus; LTx, liver transplantation; MIZ, mizoriline; MMF, mycophenolate mofetil; PSL, prednisolone.

Evaluation of ABO Antibody Titers

Among the 75 subjects who underwent ABO-I LTx without retransplantation, we evaluated antidonor ABO antibody titers in 35 whose blood type was A or B. In the remaining 40 patients, whose blood type was O, we evaluated both antidonor and

anti-nondonor ABO antibody titers. We also evaluated antidonor and anti-nondonor ABO antibody titers in 6 patients who were retransplanted with blood-type-compatible grafts. ABO antibody titers were measured by microhemagglutination assay, as previously described.¹⁸

For assay of immunoglobulin M (IgM) titers, serum samples were diluted with saline and then incubated with red blood cells at room temperature for 15 minutes. For immunoglobulin G (IgG) assays, serum samples were preincubated with 0.01 M dithiothreitol 37°C for 30 minutes to destroy IgM and then

TABLE 2.
Background data on patients in the 3 age groups at the time of transplantation

	<1 (n=16)	≥1 to <16 (n=12)	≥16 (n=47)
Demographic characteristics			
Years after LTx	7.3 (3.1–16.8)	6.6 (1.2–14.7)	5.8 (1.1–14.4)
Sex (female)	10 (62.5%)	6 (50.0%)	28 (59.6%)
Original disease			
Biliary atresia	13 (81.3%)	11 (91.7%)	6 (12.8%)
Acute liver failure	1 (6.3%)	1 (8.3%)	1 (2.1%)
Viral hepatitis	0 (0%)	0 (0%)	26 (55.3%)
Primary biliary cholangitis	0 (0%)	0 (0%)	7 (14.9%)
Alcoholic liver cirrhosis	0 (0%)	0 (0%)	2 (4.3%)
Others	2 (12.5%)	0 (0%)	5 (10.6%)
Perioperative background			
Preoperative administration of rituximab	0 (0%)	4 (33.3%)	35 (76.1%)
Splenectomy	0 (0%)	0 (0%)	27 (57.5%)
Infusion therapy	2 (12.5%)	6 (50.0%)	44 (93.6%)
Donor-to-recipient blood type			
A to O	4 (25.0%)	3 (25.0%)	18 (38.3%)
A to B	1 (6.3%)	2 (16.7%)	5 (10.6%)
B to O	7 (43.8%)	1 (8.3%)	5 (10.6%)
B to A	2 (12.5%)	0 (0%)	6 (12.8%)
AB to O	0 (0%)	0 (0%)	2 (4.3%)
AB to A	2 (12.5%)	1 (8.3%)	6 (12.8%)
AB to B	0 (0%)	5 (41.7%)	5 (10.6%)
Rejection episode			
ACR	10 (62.5%)	8 (66.7%)	21 (44.7%)
AMR	0 (0%)	1 (8.3%)	6 (12.8%)
Laboratory data at outpatient visit for last titer examination			
Aspartate transaminase, IU/L	29 (16–69)	30 (15–68)	23 (14–96)
Alanine transaminase, IU/L	18 (9–67)	22 (13–64)	16 (7–137)
Gamma-glutamyltransferase, IU/L	14 (8–115)	17 (10–116)	46 (11–538)
Total bilirubin, mg/dL	0.6 (0.4–2.0)	0.6 (0.3–1.6)	0.8 (0.1–3.7)
Bile acid, mmol/L	19 (4–119)	16 (3–209)	11 (2–152)
Immunosuppression at outpatient visit for last titer examination			
CNI (tacrolimus/cyclosporin) only	12 (75.0%)	6 (50.0%)	19 (40.4%)
CNI + MMF	2 (12.5%)	5 (41.7%)	15 (31.9%)
CNI + PSL	1 (6.3%)	0 (0%)	3 (6.4%)
CNI + MMF + PSL	0 (0%)	1 (8.3%)	7 (14.9%)
CNI + MIZ + PSL	1 (6.3%)	0 (0%)	0 (0%)
CNI + rapamycin	0 (0%)	1 (8.3%)	1 (2.1%)
MMF without CNI	0 (0%)	0 (0%)	1 (2.1%)

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CNI, calcineurin inhibitor; LTx, liver transplantation; MIZ, mizoriline; MMF, mycophenolate mofetil; PSL, prednisolone.

TABLE 3.
Backgrounds of 7 patients with antibody-mediated rejection

Case	Sex	Original disease	Age at LTx, y	Recipient blood type	Donor blood type	AMR diagnosis	Treatment	Peak titer before operation			Titer at AMR onset			Final titer		
								Anti-A IgM	Anti-A IgG	Anti-B IgM	Anti-B IgG	Anti-A IgM	Anti-A IgG	Anti-B IgM	Anti-B IgG	Anti-B IgG
1	M	LC (HBV)	50	O	A	IHBD	Steroid pulse	256	2048	128	512	32	512	32	4	8
2	M	LC (HCV)	48	O	B	IHBD	Steroid pulse	64	128	32	64	8	32	128	1024	4
3	M	LC (HCV)	51	O	A	IHBD	Steroid pulse	4	8	8	16	32	32	2	0	32
4	F	PBC	41	A	B	IHBD	Steroid pulse	NM	NM	64	4	2048	NM	NM	4	0
5	F	PBC	56	O	B	Pathological AMR	Steroid pulse	128	512	256	1024	16	512	64	128	4
6	F	Alcoholic LC	47	O	A	Pathological AMR	Steroid pulse	256	512	128	128	128	128	4	16	64
7	F	BA	4	O	A	Hepatic necrosis	Retransplantation	512	512	256	512	2048	1024	32	8	16

AMR, antibody-mediated rejection; BA, biliary atresia; HBV, hepatitis B virus; HCV, hepatitis C virus; IgG, immunoglobulin G; IgM, immunoglobulin M; IHBD, intrahepatic bile duct dilatation; LC, liver cirrhosis; LTx, liver transplantation; NM, not measured; PBC, primary biliary cholangitis.

serially diluted by 2 and incubated at 37°C for 30 minutes with red blood cells and antihuman globulin. The inverse of the maximum dilution factor at which red blood cells agglutinated was taken as the IgM or IgG antibody titer. Given that 98.8% of all Japanese people with blood type A are type A1,¹ we did not distinguish between A1 and A2.

Titer assays were performed before, and within 1 month after, transplantation. We also examined the ABO antibody titers during August through December 2012 for long-term follow-up.

Statistical Analysis

All data were analyzed by using GraphPad Prism 6; *P* less than .05 was considered statistically significant. Analysis of variance, Fisher exact test, or the Mann-Whitney *U* test was used for comparisons between groups.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki following approval from our ethics panel (approval number E2437).

RESULTS

Patient Demographics

The demographics of the study population are shown in Table 1. The median time since surgery was 6.2 years. Of the 75 patients who underwent ABO-I LTx without retransplantation, 17 were blood-type A, 18 were blood-type B, and 40 were blood-type O.

The characteristics of the 75 patients who survived after single ABO-I LTx are shown in Table 1. The patients were allocated to 3 groups according to their age at the time of transplantation: <1 year (*n* = 16), ≥1 to <16 years (*n* = 11), and ≥16 years (*n* = 48).¹ The features of each group are shown in Table 2.

Postoperative Graft Rejection

Postoperatively, 39 patients developed acute cellular rejection and 7 developed AMR (Table 3). All episodes of AMR occurred during the perioperative period. Antibody-mediated rejection did not occur after the perioperative period in ABO-I LTx patients even though they have received nothing stronger than “standard” levels of immunosuppression therapy.¹⁹

Among the 7 patients with AMR, 6 were older than 16 years. The diagnosis of AMR was hepatic necrosis in 1 patient, intrahepatic biliary complication in 4 patients, and pathological AMR (periportal edema and necrosis with C4d positivity) in 2 patients. The patient with hepatic necrosis required retransplantation, and the 4 patients with intrahepatic biliary complication required intensified immunosuppression and radiological intervention for biliary stenosis. All 7 of the patients with AMR had increased antidonor ABO antibody titers at AMR; they decreased during long-term follow-up.

Changes of Anti-ABO Antibody Titers

Antidonor ABO antibody titers in the perioperative period and at long-term follow-up are shown in Figure 2. Both IgM and IgG antidonor antibody titers against A and B antigens at long-term follow-up after LTx were significantly lower than those preoperation (*P* < .0001).

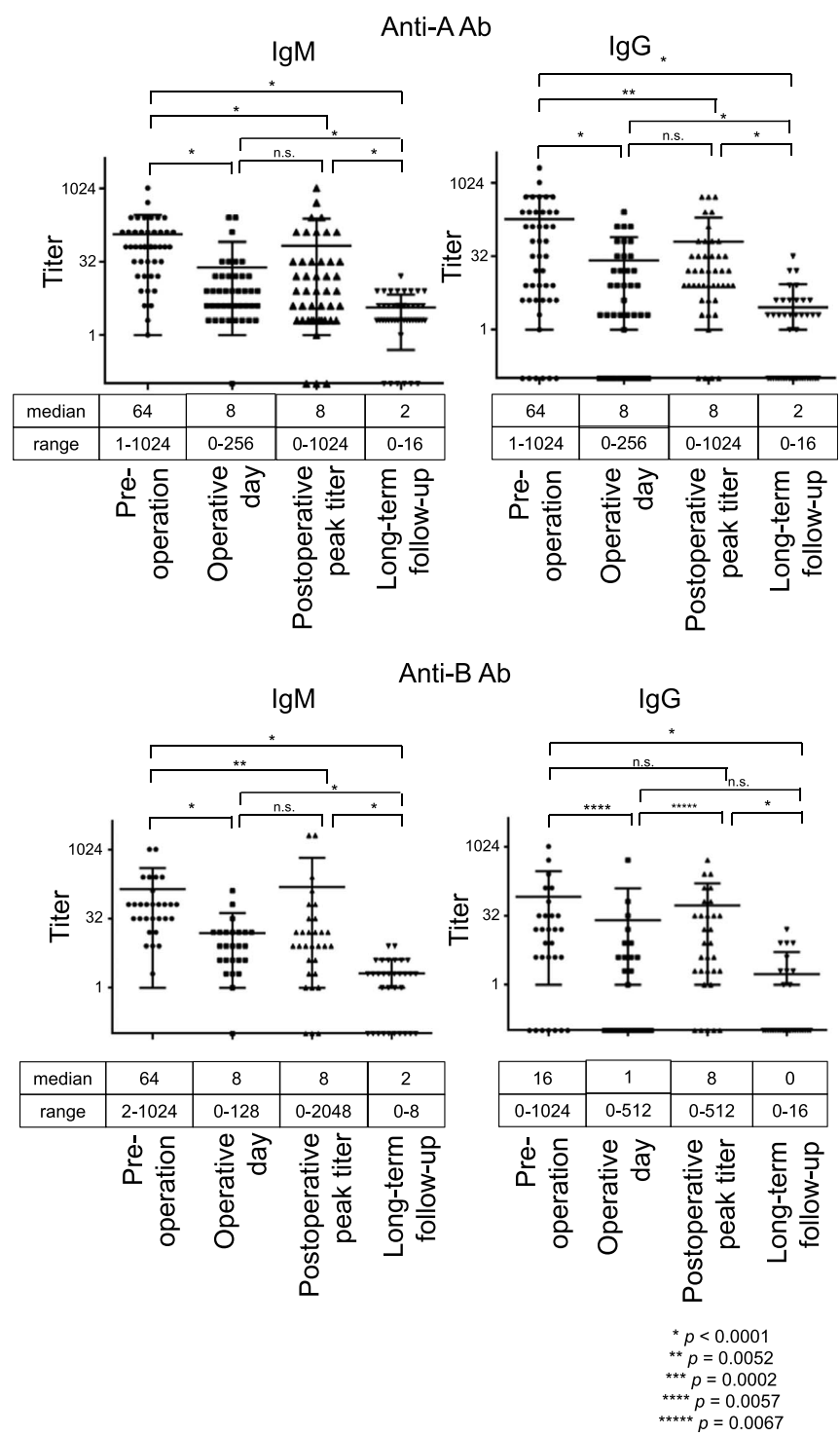


FIGURE 2. Antidonor ABO antibody titers in the perioperative period and at long-term follow-up. Antibody (Ab) titers against A and B antigens are shown separately. All anti-A, anti-B, immunoglobulin M (IgM) and immunoglobulin G (IgG) and IgG titers were significantly lower at long-term follow-up after liver transplantation (LTx) than before surgery (* $P < .0001$).

We evaluated antidonor and anti-nondonor ABO antibody titers in the 40 patients with blood type O (Figure 3). In all cases, anti-nondonor ABO antibody titers did not decrease over time and were significantly higher than antidonor ABO antibody titers in this patient subgroup (anti-A IgM, $P < .0001$; anti-A IgG, $P < .0001$, anti-B IgM, $P < .0001$; anti-B IgG, $P < .0001$).

Comparison Among Age Groups

We compared IgM and IgG antidonor ABO antibody titers during long-term follow-up among the 3 patient groups (Figure 4). Median antidonor ABO antibody titers are as follows: anti-A IgM/IgG; 4/0, anti-B IgM/IgG; 0/0 in infant, anti-A IgM/IgG; 6/0, anti-B IgM/IgG; 1/0 in the ≥ 1 to <16 years age group, and anti-A IgM/IgG; 2/2, anti-B IgM/IgG; 2/0 in

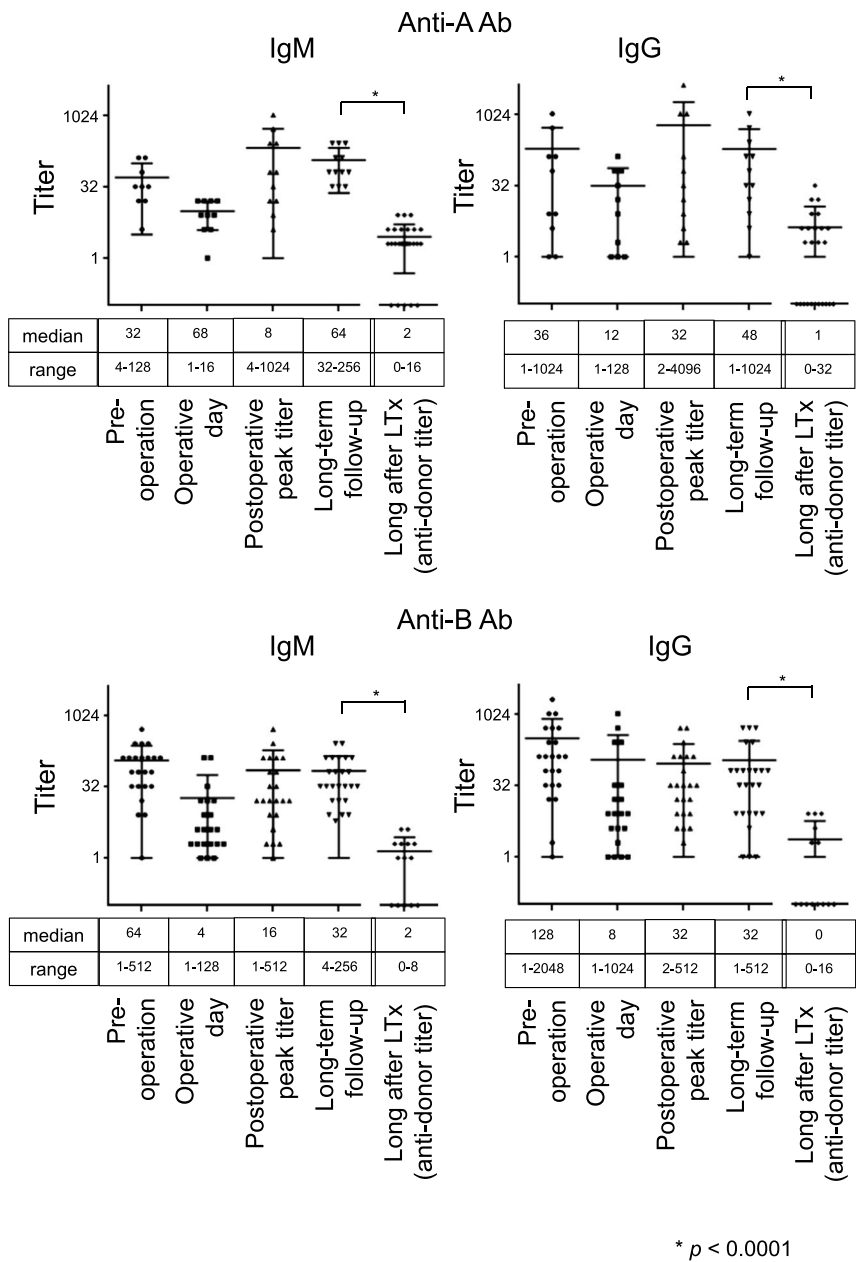


FIGURE 3. Anti-nondonor ABO-blood-type antibody (Ab) titers in the 40 recipients with blood-type O in the perioperative period and at long-term follow-up, and antidonor ABO-blood-type antibody titers at the time of long-term follow-up. Antibody titers against A and B antigens are shown separately. Anti-nondonor ABO immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody titers at long-term follow-up did not differ from those before transplantation, whereas antidonor ABO-blood type titers were significantly lower at follow-up (* $P < .0001$).

patients 16 years or older. The anti-A IgG antibody levels in patients younger than 16 years was significantly lower than that in patients 16 years of age or older (<1 year: $P = .0038$; ≥ 1 to <16 years: $P = .0230$).

We compared antidonor ABO and anti-nondonor ABO antibody titers among the 3 groups of patients with blood-type O (<1 year old, $n = 11$; ≥ 1 to <16 years, $n = 4$; ≥ 16 years, $n = 25$). In patients who were younger than 1 year, antidonor ABO antibody titers were significantly lower (IgM, $P = .0002$; IgG, $P = .0213$) than anti-nondonor ABO antibody titers. In patients who were 1 year or older to younger than 16 years, antidonor ABO IgM antibody titers were significantly lower ($P = .0286$) than anti-nondonor

ABO IgM levels; IgG titers were similar between antibody types ($P = .1429$). In patients 16 years or older, both IgM and IgG antidonor ABO antibody titers were significantly lower than anti-nondonor ABO titers ($P < .0001$ for both comparisons).

Antidonor ABO antibodies were not detected in 11 patients; 7 of these patients were younger than 1 year at transplantation, 2 patients were 1 year or older to younger than 16 years, and 2 patients were 16 years or older. The incidence of zero titer differed significantly among age groups ($P = .0006$). The recipient's blood type was A in 4 cases, B in 1 case, and O in the remaining 6 cases; blood type had no effect on the incidence of zero titer of antidonor antibodies.

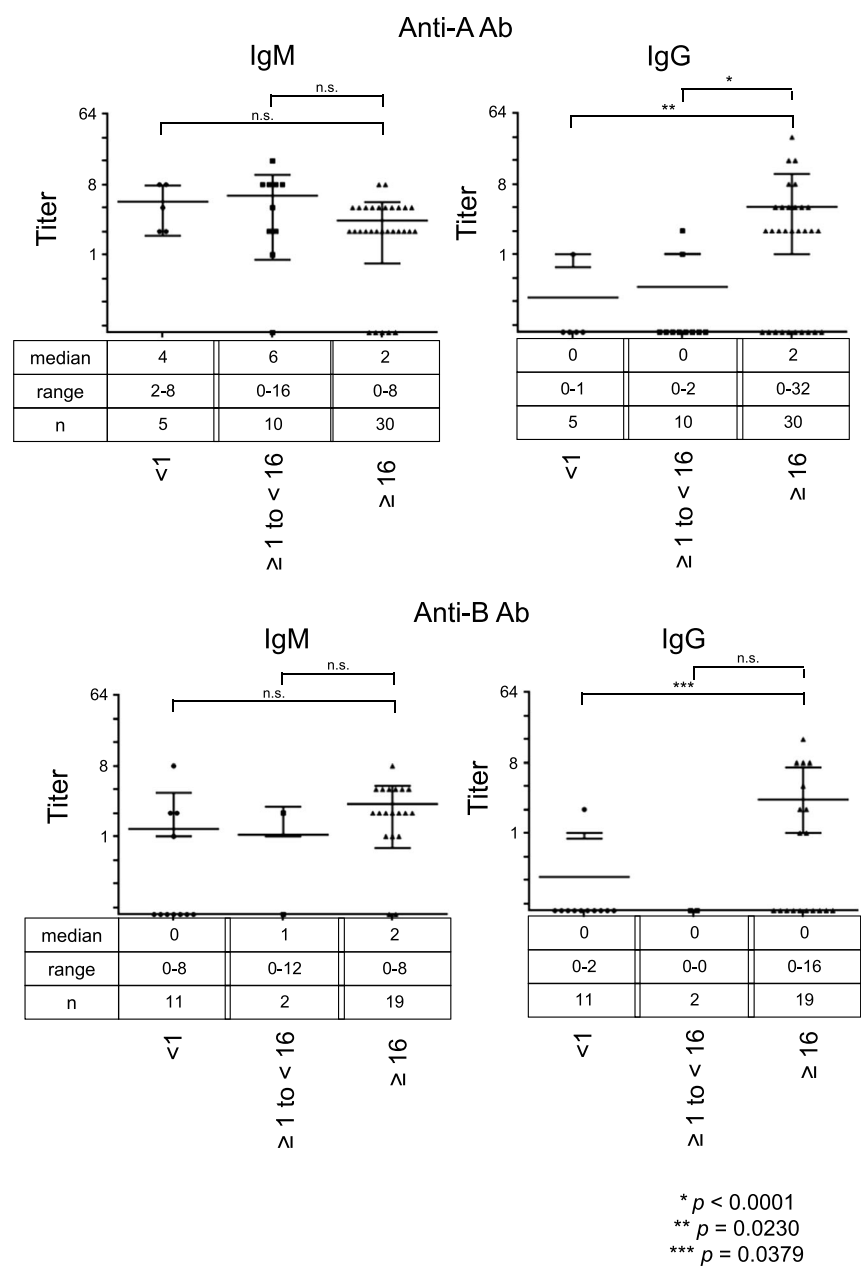


FIGURE 4. Antidonor ABO-blood type antibody (Ab) titers at long-term follow-up after liver transplantation (LTx), according to patient age at the time of incompatible LTx (I LTx) (<1 year, ≥1 to <16 years, and ≥16 years). Anti-A and anti-B immunoglobulin G (IgG) titers were significantly higher in patients 16 years of age or older than in the other 2 groups.

Study of the 6 Patients Who Underwent Retransplantation

The characteristics of the 6 patients who underwent retransplantation with ABO-compatible/-identical LTx are shown in Table 4. The cause of the graft loss was de novo autoimmune hepatitis, hepatic necrosis, or portal vein thrombosis in 1 patient each, and chronic rejection in the remaining 3 patients. The median age of these 6 participants at the time of ABO-I LTx was 2.8 years (range, 0.6–56 years), and that at the time of retransplantation was 6.6 years (range, 1.72–60.1 years). The median interval between the 2 surgeries was 3.4 years (range, 0.8–9.6 years).

In these 6 retransplanted patients, antidonor ABO antibody titers at long-term follow-up were significantly

lower than peak titers before the first transplantation ($P = .0313/.0313$; IgM/IgG) (Figure 5). In contrast, anti-donor ABO antibody titers did not differ between these time points. At long-term follow-up of these 6 patients, IgG titers were almost zero, whereas IgM titers ranged from 4 to 32 (Table 4).

We compared the antidonor ABO antibody titers at last follow-up between the 75 patients with single ABO-I LTx transplantation and the 6 patients with retransplantation. IgM antibody titers were significantly lower in the 75 patients without retransplantation than in the 6 patients with retransplantation ($P < .0001$); IgG titers did not differ between groups.

TABLE 4.

Background data and changes in antibody titers in the 6 patients who underwent retransplantation

Case	Sex	Original disease	Age, y		Cause of graft loss	Blood type	Donor blood type		First graft survival, y	Follow-up period after second LTx, y	Peak titer against blood type before first transplantation		Peak titer against targeted blood type after first transplantation		Final titer against targeted blood type		Peak titer against nontargeted blood type before first transplantation		Final titer against nontargeted blood type	
			First LTx	Second LTx			First LTx	Second LTx			IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	F	BA	15.6	25.2	De novo ALH	O	A	O ^a	9.6	2.6	64	64	64	64	4	0	32	64	64	128
2	F	BA	4.5	6.4	Hepatic necrosis (AMR)	O	A	O ^a	1.9	15.4	512	512	2048	1024	32	8	256	512	2048	64
3	M	BA	0.6	6.8	Portal vein thrombosis	A	B	A	6.2	8.3	16	4	0	0	8	0	-	-	-	-
4	F	BA	0.9	1.7	Chronic rejection	A	AB	O	0.8	11.9	64	4	4	2	16	0	-	-	-	-
5	F	HCV LC	56.1	60.1	Chronic rejection ^b	B	A	O	4.1	2.9	128	16	4	8	16	2	-	-	-	-
6	M	BA	1.2	3.7	Chronic rejection ^c	O	A	O	2.6	12.9	256	64	4	16	32	8	256	128	2	4

^a Deceased donor.

^b IFN-related chronic rejection.

^c Chronic rejection after cessation of immunosuppression for posttransplantation lymphoproliferative disease.

ALH, autoimmune hepatitis; AMR, antibody-mediated rejection; ALH, autoimmune hepatitis; AMR, antibody-mediated rejection; ALH, autoimmune hepatitis; AMR, antibody-mediated rejection; BA, biliary atresia; HCV, hepatitis C virus; IgG, immunoglobulin G; IgM, immunoglobulin M; LC, liver cirrhosis; LTx, liver transplantation.

DISCUSSION

This study revealed that antidonor ABO antibody titers were low in the long-term follow-up period after ABO-I LTx. The longest follow-up period was prolonged by 5 years since our first report in 2004.¹ Our current series of patients demonstrates 2 types of hyporesponsiveness. One—known as accommodation—is the condition characterized by lack of clinical signs despite noteworthy antibody levels. In the other condition (zero antibody status), no antidonor ABO antibodies are produced at all. In our 40 patients with blood-type O, anti-nondonor ABO antibody titers did not decrease over time and were significantly higher than antidonor ABO antibody titers. In addition, antidonor ABO antibody titers at long-term follow-up were significantly lower in pediatric patients than in adult patients. Compared with those in the 75 patients without retransplantation, antidonor ABO IgG antibody titers in the 6 retransplanted patients remained low at long-term follow-up, but IgM titers were increased slightly after the removal of ABO-incompatible liver grafts.

The 2 forms of accommodation include one in which antibodies bind to antigens and one in which the antibodies do not bind. In the setting of antigen-antibody binding, 3 mechanisms can be proposed: weak complement activation (such as decreased affinity of the IgG2 IgG4 subset); the presence of cytoprotective factors in the endothelium (such as CD55, CD59, and HO-1)²⁰; and the repair of tissue injury. In the absence of antigen-antibody binding, loss of antigenicity may be the main mechanism.²¹

One potential mechanism of zero antibody status is the absence of B cells capable of producing antidonor antibodies; malfunction of those B cells is an alternative potential mechanism. Clonal deletion in children after heart transplantation or LTx has been reported.^{5,22} Furthermore, long-lived plasma cells in the bone marrow and spleen likely are involved in persistent antibody production against specific antigens.²³ Because of their low frequency in the peripheral blood, we were unable to examine these plasma cells (data not shown).

In the 6 patients that underwent retransplantation, antidonor ABO antibody titers remained low at long-term follow-up. However, these antidonor IgM antibody titers were significantly higher than those of the 75 patients with single transplantations. These findings suggest that continuous presentation of antigens—or at least the presence of antigen—is necessary for the maintenance of hyporesponsiveness.

This study had several limitations. First, it was a retrospective study that included patients who underwent transplantations over a long period, and differences in their treatments may have influenced outcomes. In addition, the duration of “long-term” varied widely. Furthermore, we were unable to investigate the kinetics of B cells and long-lasting plasma cells.

In conclusion, donor-specific hyporesponsiveness occurred after ABO-I LTx during long-term follow-up, particularly in pediatric patients. Prolonged persistence of blood antigens might contribute to this hyporesponsiveness.

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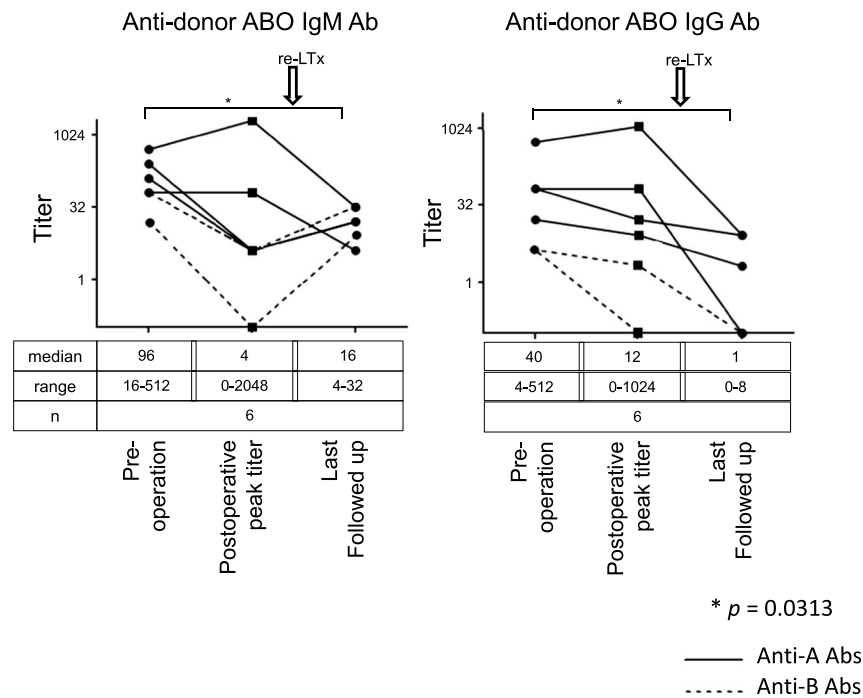


FIGURE 5. Changes in antidonor ABO antibody (Ab) titers in 6 patients with retransplantation. Peak titers before and after the first (ABO-incompatible) transplantation and titers at long-term follow-up after retransplantation (ABO-compatible/-identical liver transplantation [LTx]) are shown. IgG, immunoglobulin G; IgM, immunoglobulin M.

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