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The Highest Anti-B Antibody Titer of 8192 in a Patient Undergoing ABO-incompatible Living-donor Liver Transplantation: How to Achieve Donor-specific B-cell Tolerance?

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Antibody-mediated rejection (AMR) is a refractory rejection after ABO blood-type incompatible (ABOi) or donor-specific antibody-positive liver transplantation.¹⁻³ AMR often results in irreversible damage not only to the hepatic parenchyma but also to intrahepatic bile ducts, thereby increasing the risk of graft failure even with appropriate treatments.⁴⁻⁶ Pretransplant desensitization using rituximab, an anti-CD20 monoclonal antibody, has significantly improved the outcomes of ABOi living-donor liver transplantation (LDLT).^{7,8} However, once AMR develops, it is often resistant to conventional treatments, leading to graft failure.⁹

High pretransplant antibody titers, especially initial titers before desensitization, are a well-known risk factor for AMR development after ABOi-LDLT, but there is limited evidence of ABOi-LDLT with high anti-donor A/B antibody titers ≥ 2048 ^{8,10-12} (Table S1, SDC, <https://links.lww.com/TXD/A792>). Here we report a case of successful ABOi-LDLT in a patient with extremely high initial anti-B IgG titer of 8192, along with 4 other cases with anti-donor A/B IgG titers ≥ 2048 experienced in the era of rituximab desensitization.

CASE PRESENTATION

A 58-y-old Japanese man (blood-type O) with exceptionally high titers of anti-B IgG: 8192 and IgM: 256 was scheduled to undergo an ABOi-LDLT from his son (type B). The indication for transplant was alcoholic cirrhosis (Model for End-stage Liver Disease score: 17) and hepatocellular carcinomas within Milan criteria. The pretransplant desensitization protocol included rituximab of 375 mg/m² 3 wk before transplant, mycophenolate mofetil (MMF) of 500–1000 mg/d for 2 wk pretransplant, and tacrolimus (target trough: 4–5 ng/mL) for a week pretransplant. Just before transplantation, plasma exchange (PEX, 1.5 times the estimated plasma volume was replaced with the same volume of fresh-frozen plasma [FFP] each time)¹³ was performed once a day for a total of 4 sessions. After 3 sessions of PEX, the anti-B IgG titer had declined steadily from an initial 8192 to 16 two days before transplant but rebounded rapidly to 128 by the next morning in only half a day. The method for measuring anti-ABO IgG and IgM titers at our institute has been described previously.¹ Although circulating

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T.T. and K.H. designed the study and wrote the article. K.H., H. Ohe, A.M., and H. Okajima participated in surgery and peritransplant patient management. K.Y. and R.H. contributed to the laboratory work. M.N. supervised laboratory works. S.U. and E.H. supervised peritransplant patient management. All authors participated in the interpretation of the data and review of the article. All authors approved the final version of the article.

This study was approved by the Ethics Committee of Kyoto University (approval number: R1473-3) and was conducted in accordance with the institutional guidelines as well as the ethical guidelines mandated by the Declaration of Helsinki (as revised in 2013).

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

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B-lymphocytes had almost completely disappeared (CD19+: 0.1% and CD20+: 0.0%), additional rituximab: 300 mg/individual was administered after the final PEX on the day before transplant to further mitigate the potential risk of post-transplant AMR (Figure 1).

The patient underwent ABOi-LDLT using a right-lobe graft with a graft-to-recipient weight ratio of 1.34%. Neither splenectomy nor local infusion therapy via the hepatic artery or portal vein was performed. Post-transplant immunosuppression included tacrolimus, MMF, and steroids as per protocol.¹

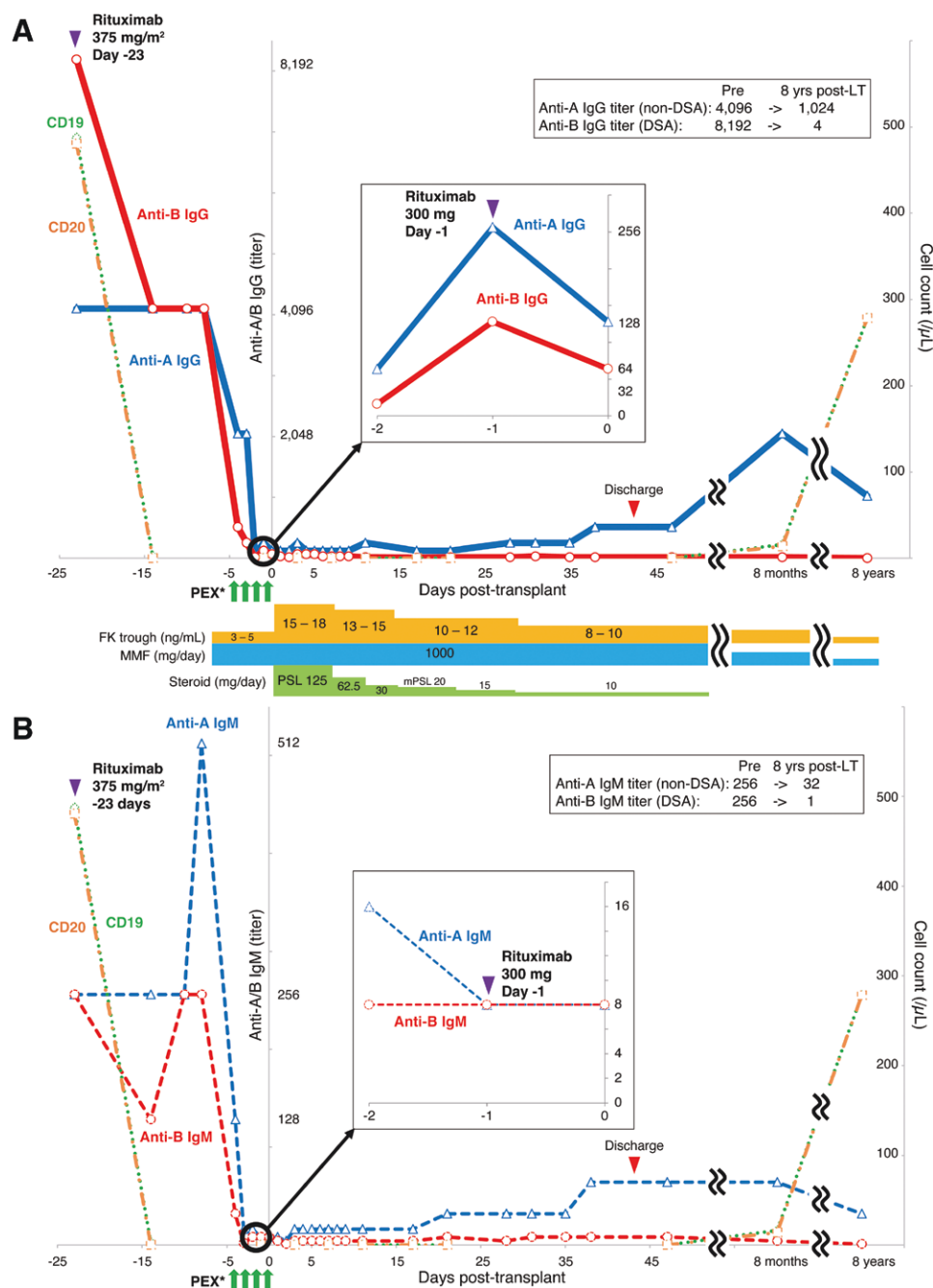


FIGURE 1. Peritransplant anti-A/-B IgG/M titers and CD19+/20+ counts. A, Following the first dose of rituximab and 3 PEX sessions, the anti-B IgG titer had declined steadily from an initial 8192 to 16 two days before transplant but rebounded sharply up to 128 the next morning. Although circulating CD19+ and CD20+ B-lymphocytes had disappeared to 0.1% and 0.0%, respectively, additional rituximab: 300 mg/individual was administered after the final PEX on the day before transplant to further reduce the risk of antibody-mediated rejection (AMR). The patient's post-transplant course has been good with no significant infections or rejections. Throughout 8 y post-transplant, his anti-B IgG/M titers have been stable at 4/<1, respectively, while anti-A IgG/M titers had recovered to 2048/32 by 8 mo post-transplant, respectively, indicating that specific immune tolerance to the donor B-antigen was successfully achieved. B, As with IgG titers, the patient's anti-B IgM titers have been stable at <1 throughout 8 y post-transplant, while anti-A IgM titers had recovered to 64 by 6 wk post-transplant, indicating that specific immune tolerance to the donor B-antigen was successfully achieved. *The 4 green arrows indicate that PEX was performed daily for 4 consecutive days until the day before living-donor liver transplantation (LDLT). DSA, donor-specific antibody; FK, tacrolimus; LT, liver transplantation; MMF, mycophenolate mofetil; mPSL, methylprednisolone; PEX, plasma exchange; PSL, prednisolone.

TABLE 1.
Our 5 cases with anti-donor A/B IgG titer ≥2048 in the era of rituximab

Case	1	2	3	4	The present case
Transplant year	2004	2006	2014	2016	2013
Sex	F	M	F	F	M
Age	58	65	66	51	58
Combination of ABO blood type	A to O	AB to O	A to O	B to O	B to O
Initial anti-donor A/B IgG titer before rituximab	2048	2048	2048	2048	8192
Anti-donor A/B IgG titer just before LT	128	32	128	128	64
Number of pre-LT PEX sessions	3	4	2	2	4
Number of post-LT PEX sessions	0	0	1	0	0
Number of pre-LT rituximab doses	1	1	1	1	2
Number of post-LT rituximab doses	0	0	0	1	0
AST/ALT on PTD-3, U/L	79/135	119/122	112/330	92/462	40/103
AST/ALT on PTD-7, U/L	88/151	139/290	66/145	13/55	25/68
AST/ALT on PTD-14, U/L	16/44	65/133	72/114	12/14	28/67
AST/ALT on PTD-30, U/L	15/15	56/80	50/81	14/31	17/18
AST/ALT on PTD-90, U/L	25/33	51/27	21/16	17/13	15/35
AMR	No	No	No	No	No
Blood culture positivity	No	No	No	No	<i>Pseudomonas aeruginosa</i> on PTD-7, cured with MEPM
CMV antigen positivity	Positive on day 28, cured by antivirals	Positive on day 52, cured by antivirals	No	Positive on day 33, cured by antivirals	Positive on day 24, cured by antivirals
Latest anti-donor A/B IgG titer	64	64	64	2	4

ALT, alanine aminotransferase; AMR, antibody-mediated rejection; AST, aspartate aminotransferase; CMV, cytomegalovirus; F, female; LT, liver transplantation; M, male; MEPM, Meropenem; PEX, plasma exchange; PTD, post-transplant day.

His post-transplant course has been good to date, with no significant infections or rejections. Throughout 8 y post-transplant, his anti-B IgG/M titers have long been stable at 4/<1, respectively, while anti-A IgG/M titers had recovered to 2048/32 by 8 mo post-transplant, respectively, indicating that donor-specific immune tolerance to the blood-type B-antigen was successfully achieved (Figure 1).

In addition to the present case, we have experienced 4 other patients to date with initial anti-A/B IgG titers of ≥2048. Of note, the recipients’ blood types were all type O (Table 1). They all received rituximab 2–3 wk pretransplant, followed by 2–4 sessions of PEX just before LDLT. All demonstrated a significant reduction in anti-donor A/B IgG titers before transplantation (Figure 2A–D). All 5 patients had favorable post-transplant courses without AMR development and successfully achieved donor-specific B-cell tolerance.

DISCUSSION

We previously reported that anti-donor A/B IgM rather than IgG is a significant risk factor for the development of AMR. This is because IgM is produced more rapidly than IgG upon sensitization and has a higher complement activation capacity than IgG on its own.¹ However, the role of anti-donor A/B IgG in AMR should not be underestimated. Among IgG subtypes, IgG1 and IgG3 have particularly high affinity for the complement component C1q,¹⁴ suggesting that anti-donor A/B IgG may contribute significantly to the onset and severity of AMR, especially in patients with extremely high IgG levels, as observed in this patient.

In the present case, despite the marked rebound of anti-B IgG from 16 to 128 within half a day after multiple PEX,

there was no corresponding increase in peripheral blood B-cell counts, suggesting that the antibodies were likely produced predominantly by long-lived plasma cells residing in the bone marrow and/or secondary lymphoid organs, such as lymph nodes, gut, or spleen. These plasma cells are resistant to rituximab-mediated depletion of B-cell lineage and may have been the primary source of persistent antibody production following the desensitization therapy.

We previously analyzed lymphocyte subsets in lymph nodes and the spleen obtained from recipients who had undergone ABOi-LDLT.¹⁵ Figure S1 (SDC, <https://links.lww.com/TXD/A792>) shows the data extracted from that study, which only included measurements of CD19⁺ and CD20⁺ B cells after rituximab administration (375 mg/m²). Rituximab pretreatment had eliminated CD19⁺ B cells from the peripheral blood; however, CD19⁺ B cells substantially remained in lymph nodes and the spleen at the time of transplantation (Figure S1A, SDC, <https://links.lww.com/TXD/A792>). Although fewer in number than CD19⁺ cells, there were several cases in which CD20⁺ B cells remained in lymph nodes at levels of ≥20% (Figure S1B, SDC, <https://links.lww.com/TXD/A792>). Similarly, a clinical study of ABOi kidney transplantation demonstrated that a single dose of rituximab effectively depleted B cells in circulating blood, but not in lymph nodes.¹⁶ These findings highlight that even though rituximab pretreatment appears to have sufficiently eliminated circulating B cell lines, substantial numbers of CD19⁺ B cell lineage or CD20⁺ long-lived plasma cells remain in lymphoid tissues or bone marrow. Therefore, in cases with significant rebound of anti-A/B antibody titers after PEX, as observed in this patient, additional rituximab administration should be considered to effectively eliminate remaining B cells in secondary lymphoid tissues, even though CD19/CD20 levels seemingly disappear from the peripheral

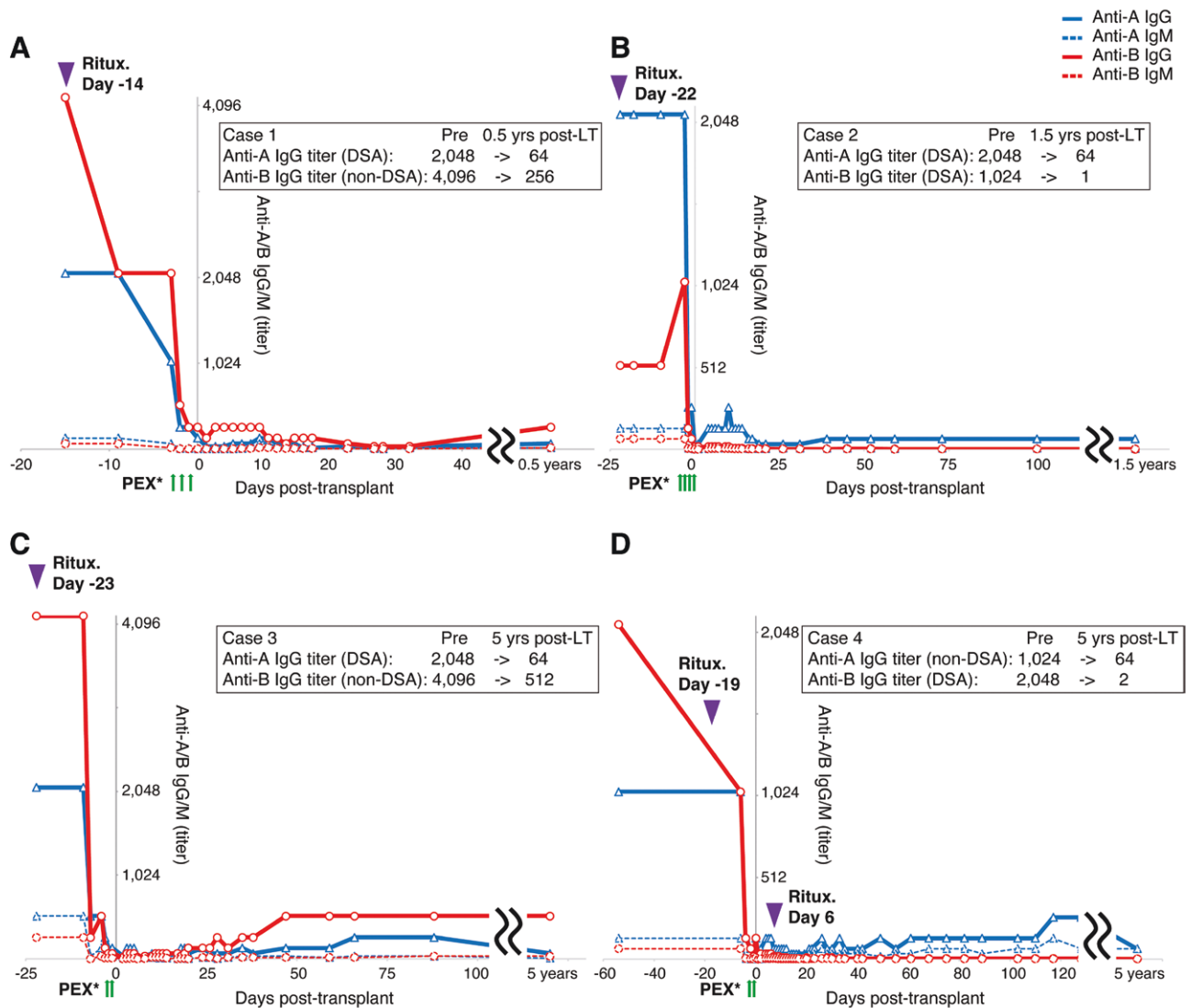


FIGURE 2. Four other cases with anti-donor A/B IgG titer ≥ 2048 in the era of rituximab. All recipients received rituximab 2–3 wk before transplantation, followed by 2–4 sessions of PEX. A significant reduction in anti-donor A/B IgG titers was observed in all the patients before transplantation (A–D). Only case 4 (D) showed a slight increase in the anti-B IgG titer post-transplant and received additional rituximab on post-transplant day 6. None of the patients have developed antibody-mediated rejection (AMR), and all achieved specific immune tolerance to anti-donor A/B antigens. Additional details are provided in Table 1. *The green arrow indicates 1 session of PEX using 1.5-fold estimated plasma volume of fresh-frozen plasma (FFP). Three green arrows indicate that PEX was performed daily for 3 consecutive days until the day before living-donor liver transplantation (LDLT). DSA, donor-specific antibody; LT, liver transplantation; PEX, plasma exchange; Ritux., rituximab.

blood. As long as a vast amount of lymphoid tissues remain in the body even after splenectomy, sufficient rituximab pretreatment may be more practical and effective than splenectomy alone. The favorable clinical course of this patient, with no significant post-transplant AMR, further supports the efficacy of this approach.

To date, only 1 patient with an anti-A IgG titer of 8192 has been reported¹¹; however, this patient's initial, pretreatment anti-A IgG titer was just 1024. It rose up to 8192 secondary to pretransplant plasmapheresis. It is presumed that plasmapheresis performed before rituximab administration led to CD20⁺ B-cell activation, resulting in secondary antibody production. This highlights the importance of timing in desensitization protocols: PEX/plasmapheresis, especially using FFP, can provoke allergic reactions¹³ and unexpected immune activation, if the preceding desensitization or concomitant

immunosuppression is insufficient. Accordingly, our protocol involves rituximab (375 mg/m²) administration 3 wk before transplant, followed by oral MMF and tacrolimus. When administering FFP to recipients undergoing ABOi-LDLT, we exclusively use FFP with blood-type AB to avoid introducing additional anti-A or anti-B antibodies and any potential influence on subsequent antibody-titer measurements. PEX is initiated only after confirming sufficient B-cell depletion and evaluating anti-A/B antibody titers. If antibody titers rebound, additional rituximab and individualized scheduling of subsequent PEX sessions are considered. This approach avoids pre-rituximab PEX and the associated risk of unintended immune activation, as reported by Shimoda et al.¹¹ We have also summarized previously reported ABOi-LDLT cases with titers of ≥ 2048 in Table S1 (SDC, <https://links.lww.com/TXD/A792>).^{8,10–12} Except for the case report by Shimoda et

al,¹¹ most of these reports lack detailed individual-level data, limiting direct comparison. Further accumulation of well-documented high-titer cases will be essential to better characterize immunological risk and refine desensitization strategies in this challenging population.

Regarding post-transplant infection surveillance, 2 sets of blood cultures were obtained during febrile episodes to identify the causative organisms, and cytomegalovirus antigen (C10–C11) was also monitored weekly until discharge. The episodes of positive blood cultures and cytomegalovirus antigenemia in these 5 cases are summarized in Table 1. In all cases, close monitoring and early therapeutic intervention successfully prevented the development of severe infections, resulting in favorable clinical outcomes for all patients.

A limitation of this study is the absence of IgG subclass-specific and C1q-binding assays, which precludes a detailed assessment of the effector function of the detected anti-B antibodies. Given the critical role of IgG1 and IgG3 in complement activation and complement-binding capacity in the pathogenesis of AMR, this limitation restricts our ability to fully characterize the immunological risk associated with high-titer anti-A/B antibodies. Future studies incorporating these functional assays will be essential to better understand the clinical significance of high-titer anti-A/B antibody profiles in ABOi-LDLT.

In conclusion, we present a successful case of ABOi-LDLT with the highest initial (pretransplant) anti-B IgG titer than ever reported. Even in patients with extremely high anti-A/B antibody titers, ABOi-LDLT can be safely performed through comprehensive pretransplant desensitization and careful peritransplant monitoring of antibody titers and B-lymphocyte counts.

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