Int Surg 2017;**102:**536–541
DOI: 10.9738/INTSURG-D-16-00246.1

Living Donor Liver Transplantation in a Highly Allo-Sensitized Recipient: Confusing Influence of Rituximab on the Lymphocytotoxicity Crossmatch Test. A Case Report

Takatsugu Matsumoto[1], Taku Aoki[1], Yukihiro Iso[1], Masato Kato[1], Kohichi Yuseki[2], Tetsuji Kobata[3], Keiichi Kubota[1]

[1]*Department of Gastroenterological Surgery, Dokkyo Medical University, Tochigi, Japan*

[2]*Clinical Laboratory Department, Dokkyo Medical University Hospital, Tochigi, Japan*

[3]*Department of Immunology, Dokkyo Medical University, Tochigi, Japan*

Objective: We report a successful living donor liver transplantation (LDLT) from donor (husband) to highly allo-sensitized recipient (wife) against donor-adopting sufficient preoperative preparation. Methods: A 47-year-old woman with primary biliary cirrhosis was referred to our hospital as a potential candidate of LDLT. Her husband was willing to donate his hemiliver. As the lymphocytotoxicity crossmatch (LCT-XM) test based on a complement-dependent cytotoxicity and flow panel reactive antibody (PRA) test revealed that the patient had strong donor-specific anti-HLA antibody, the patient received rituximab twice for preoperative desensitization. A total of 5 rounds of plasmapheresis were also performed. Results: Nevertheless, the LCT-XM test 9 days after the administration of rituximab did not turn to negative while flow PRA test was almost negative. Suspecting that residual rituximab in the recipient's serum might interfere with the LCT-XM test because of its potential ability to activate the complement, we retried the test after absorbing rituximab from the serum with immunomagnetic bead. Conclusion: The result: The LCT-XM test turned to negative, suggesting that the desensitization therapy was adequate. A left liver graft was transplanted as planned, and the postoperative course was uneventful. The patient is doing well 12 months after transplantation.

Key words: Living donor transplantation – Lymphocytotoxic crossmatch – Rituximab

Corresponding author: Takatsugu Matsumoto, MD, Department of Gastroenterological Surgery, Dokkyo Medical University, 880 Kitakobayashi, Mibu, Tochigi 321-0293, Japan.
Tel.: +81 282 87 2158; Fax: +81 282 86 6317; E-mail: t-matsu@dokkyomed.ac.jp

Table 1 Laboratory data on admission[a]

AST	(8–38, U/l)	172	WBC	(3,200–8,500, /mm^3)	6,200
ALT	(4–44, U/L)	89	Hb	(11.0–14.8, g/dL)	6.8
ALP	(104–338, U/L)	2,804	Plt	(16.4–35.8, 10^4/μL)	12.6
LDH	(106–211, U/L)	192			
GGTP	(16–73, U/L)	294	PT	(70-, %)	79
T-Bil	(0.1- 1.0, mg/dL)	6.8	APTT	(−31.6, s)	47.5
TBA	(−10.0, μmol/L)	168.2	Fbg	(150–400, mg/dL)	311
TP	(6.5–8.1, g/dL)	7.8			
Alb	(3.9–4.9, g/dL)	2.2	CRP	(−0.3, mg/dL)	0.45
			IgG	(680–1620, mg/dL)	2,754
Na	(135–151, mEq/L)	132	IgM	(57–288, mg/dL)	715
K	(3.3–4.8, mEq/L)	3.8	IgA	(84–438, mg/dL)	406
Cl	(98–108, mEq/L)	99	IgE	(−174, mg/dL)	329.4
UN	(7–21, mg/dL)	11	AMA	(−1:20, titre)	160
Cre	(0.4–0.8, mg/dL)	0.44	Cu	(68–128, mcg/dL)	263

[a]Underlines show abnormal data.

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; Cre, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Fbg, fibrinogen; GGTP, gamma-glutamyltransferase; Hb, hemoglobin; Plt, platelet; PT-INR, prothrombin time-international normalized ratio; T-Bil, total bilirubin; TP, total protein; UN, urea nitrogen.

There is established evidence to suggest a negative impact on graft outcome after ABO-incompatible liver transplantation (LT) because of an increased risk of graft loss through antibody-mediated rejection.[1] On the other hand, it remains controversial whether a positive crossmatch result for anti-human leucocyte antigen (HLA) antibodies has a negative impact on graft outcome in LT.[2–4] Recent studies have revealed that highly sensitized recipients show poorer engraftment and survival than nonsensitized recipients.[5] Because of the severe shortage of deceased graft donors in Japan, the majority of liver grafts are obtained from living donor relatives and spouses. It is known that bidirectional cell transfer during pregnancy induces allo-sensitization between the mother and her offspring or between the spouses,[6] hence, the need for greater concern about the presence of donor-specific antibodies in cases of interfamilial living donor liver transplantation (LDLT). To overcome this problem, preoperative desensitization therapy has been applied, and good results have been reported.[7] Here, we report a case of successful LDLT using a familial liver graft in which a preoperative lymphocytotoxic crossmatch (LCT-XM) test based on complement-dependent cytotoxicity gave a highly positive result. This case draws attention to the potentially confusing impact of rituximab on the direct crossmatch test and to the method for overcoming such problems.

Case Report

The recipient was a 47-year-old woman with end-stage liver disease due to primary biliary cirrhosis (PBC). According to the laboratory data on admission (Table 1), her model for end-stage liver disease (MELD) score was 15. She had had 3 episodes of delivery. She was considered to be a candidate for liver transplantation because of her high MELD score. Due to the severe shortage of deceased donor grafts in Japan, we planned living donor liver transplantation (LDLT), and her husband stated a willingness to donate his hemiliver. The ABO blood type was identical, but the LCT-XM test as a direct crossmatch test revealed that the pretransplant serum of the recipient had strong anti-B-lymphocytotoxicity against her husband. In addition, a flow panel reactive antibody (PRA) test revealed that she had donor-specific anti-HLA antibody (DSA) and its allo-specificity was HLA-B55, with 6 HLA mismatches (Table 2). The normalized mean fluorescence intensity (MFI) was 11,658. After obtaining written informed consent from the patient and donor, then waiting for the approval of the intra-institutional committee, we began preoperative preparations.

For preoperative desensitization, rituximab (375 mg/m^2 body surface area) was injected twice after the initial direct crossmatch test. A total of 5 rounds of plasmapheresis were also performed. A flow PRA

Table 2 HLA characteristics of the recipient and donor[a]

	A locus		*B locus*		*DR locus*		*C locus*		*DQ locus*	
Recipient	24	24	61	52	4	15	w10	w12	4	6
Donor	2	2	48	55	8	15	w1	w10	8	6

[a]Underlines show mismatched HLA loci.

test performed after the desensitization therapies showed a marked decrease of DSA in her serum (Fig. 1). However, the result of a second LCT-XM test was still strongly positive for B cells. The interval between the start of rituximab administration and the second crossmatch test was 9 days. As we suspected interaction between residual rituximab in the recipient's serum and the donor's B lymphocytes, a direct crossmatch test was performed again using immunomagnetic separation method. Briefly, the magnetic beads coated with the goat anti-mouse IgG antibody were added to the serum so that the beads could bind with rituximab, which is a chimeric monoclonal antibody composed of human and murine immunoglobulins. Then, the immune complex was removed by the magnet, and

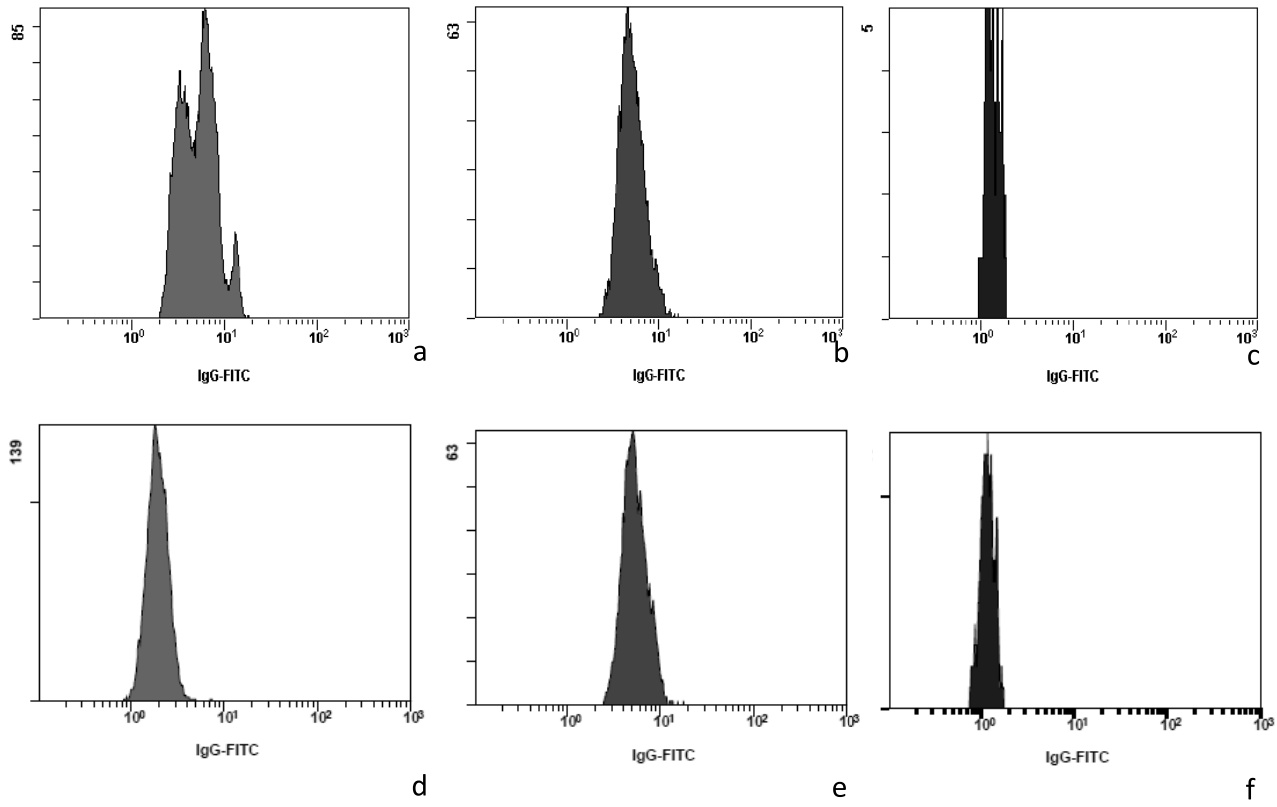

Fig. 1 Flow-panel reactive antibody (PRA) tests before and after desensitization therapies. Upper panels (a, b, and c) show the results of the first flow panel-reactive antibody test. Lower panels (d, e, and f) show the results of the flow PRA test after desensitization therapies. (a) HLA class I histogram before sensitization therapies, showing multiple peaks which indicating that the serum of the recipient contains anti-HLA class I antibody. (b) Control histogram before desensitization therapies. (c) HLA class II histogram before desensitization therapies, showing a single smooth peak, indicating that serum of the recipient contains no anti-HLA class II antibody. (d) HLA class I histogram after desensitization therapies, showing a single homogeneous peak indicating that the amount of the antibody in the patient's serum is markedly decreased. (e) Control histogram after desensitization therapies, showing no alteration compared with the histogram before the therapies. (f) HLA class II histogram after desensitization therapies, showing no alteration compared with the histogram before the therapies.

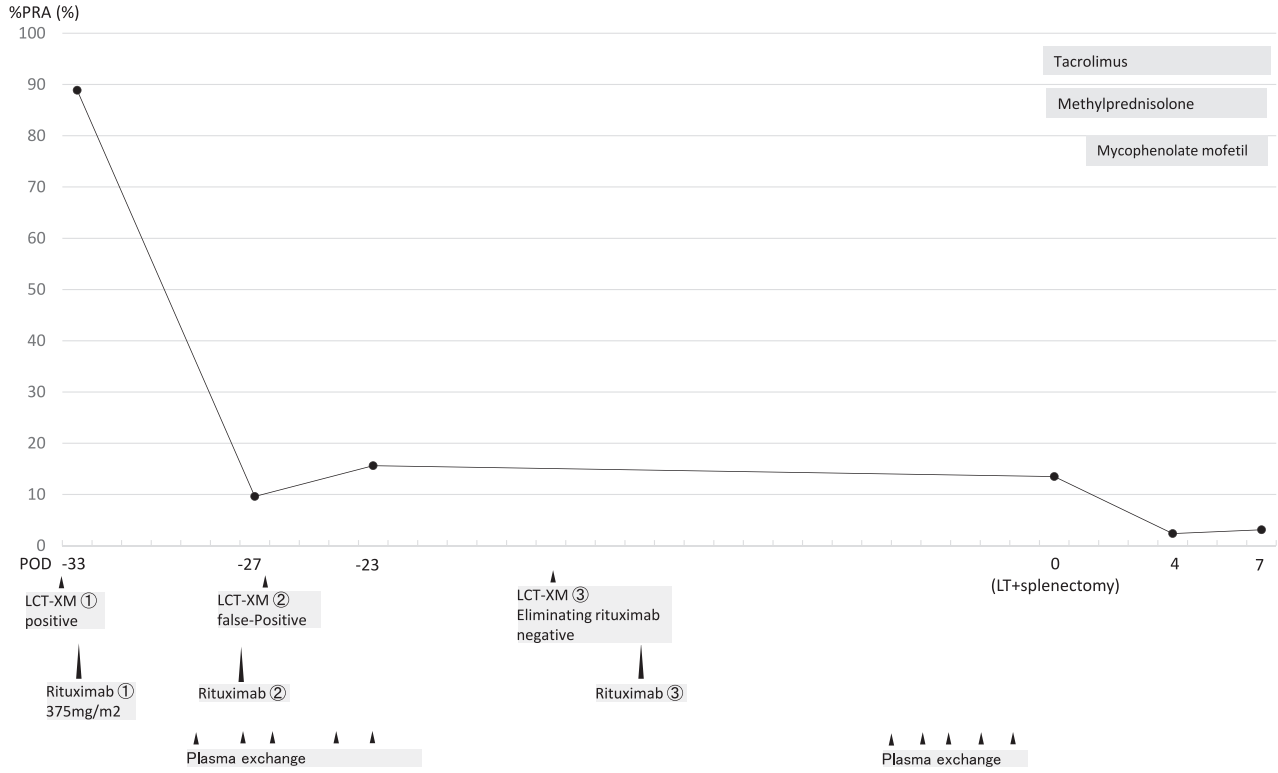

Fig. 2 Clinical profile of the present patient. LCT-XM, lymphocytotoxicity crossmatch test; LT, liver transplantation; PRA, panel reactive antibody.

rituximab was eliminated from the recipient's sera. Thereafter, the result of the direct crossmatch test turned negative, revealing that our preoperative desensitization therapy had been adequate and that rituximab had been responsible for the false positive result of the second crossmatch test. Because of the false-positive result of the direct crossmatch test done after the first desensitization therapy, we could not perform LDLT as per the planned schedule. In view of the interval between the desensitization therapy and transplantation, additional rituximab administration and plasmapheresis were performed. The left liver graft was then finally implanted. Splenectomy was also performed during the LDLT, expecting the elimination of the B lymphocytes stored in the spleen. Moreover, to perform local graft infusion therapy, a catheter was inserted in the portal vein for infusion of heparin, methylprednisolone, and prostaglandin E. For post-transplant immunosuppression, methylprednisolone and tacrolimus were administered at the normal doses. In addition, mycophenolate mofetil (MMF; 2,000 mg/d) was started on postoperative day 5. The postoperative course was uneventful without signs of either antibody-mediated rejection or acute cellular rejection. A flow PRA test performed 1 week after LDLT showed no increase in the DSA level. The clinical course is summarized in Fig. 2. The patient was discharged on postoperative day 30. She has since been well with good graft function during 12 months of follow-up. As she showed no suspicion of DSA-caused graft injury, biopsy has not been performed after LDLT.

Discussion

In contrast to renal transplantation, the effect of preoperative desensitization therapy on highly allo-sensitized recipients in liver transplantation remains controversial, especially in cases of deceased donor liver transplantation.[2–4] However, recent studies have indicated that direct crossmatch-positive LDLT contributes to the development of hyperacute or acute rejection and subsequent graft loss.[5,8,9] To prevent antibody-mediated rejection, it is important to deplete B cells and antibodies. In our patient, rituximab and plasmapheresis were employed as desensitization treatments.

Rituximab, a high-affinity CD20-specific antibody, plays an important role in desensitization for organ transplantation. There are 3 postulated mechanisms of action of rituximab for B-cell depletion: complement-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity, and induction of apoptosis. *In vivo*, it has been reported that the first mechanism is dominant[10] because rituximab contains a complement-activating isotype human IgG1. After rituximab administration, depletion of B cells is usually confirmed within 1 week.

In ABO-incompatible liver transplantation, crossmatch testing is useful for assessing the effect of desensitization, as the results are simple and quantitative. However, in the case of highly allo-sensitized recipients pretreated with rituximab, instead of DSA, residual rituximab in the serum would kill donor B cells in the direct crossmatch test. Consequently, rituximab would produce a false-positive B-cell crossmatch result. Therefore, in such cases, it is difficult to estimate the effect of desensitization therapy by direct crossmatch testing. Since the mean half-life of rituximab is reported to be 59.8 hours,[11] the false-positivity rate is assumed to be high when the interval between the administration and the test is short. There are new techniques for eliminating the effect of rituximab when performing the direct crossmatch test.[12,13] In renal transplantation, Ishizuka *et al* reported the effectiveness of protease for removal of the cell surface CD20 and FcR (binding with anti-CD20 Ab).[14] Although this method is effective for flow cytometry crossmatch testing, it is not applicable to direct crossmatch testing because protease destabilizes the cell membrane during removal of cell surface antigen, and this leads to an incorrect test result. In the present case, we used immunomagnetic bead absorption for removal of the circulating rituximab.[15,16] Immunomagnetic beads coated with anti-mouse Fab antibody efficiently absorb circulating rituximab, and thus the false-positive reaction was completely abolished, allowing us to successfully confirm that the DSA had been depleted and that our preoperative desensitization therapy had been satisfactory.

In conclusion, we have successfully performed LDLT in a highly allo-sensitized recipient as a result of sufficient preoperative preparation. It should be considered that rituximab may induce a false-positive result in the LCT-XM test. The use of immunomagnetic beads to eliminate interference from rituximab in the direct crossmatch test might be of value for estimating the real effect of desensitization therapies.

Acknowledgments

We have no conflicts of interest to declare, we received no funding/grant support for this study.

References

1. Egawa H, Teramukai S, Haga H, Tanabe M, Fukushima M, Shimazu M. Present status of ABO-incompatible living donor liver transplantation in Japan. *Hepatology* 2008;**47**(1): 143–152
2. Navarro V, Herrine S, Katopes C, Colombe B, Spain CV. The effect of HLA class I (A and B) and class II (DR) compatibility on liver transplantation outcomes: an analysis of the OPTN database. Liver Transplant 2006;**12**(4):652–658
3. Markus BH, Duquesnoy RJ, Gordon RD, Fung JJ, Vanek M, Klintmalm G *et al*. Histocompatibility and liver transplant outcome. Does HLA exert a dualistic effect? *Transplantation* 1988;**46**(3):372–377
4. Donaldson P, Underhill J, Doherty D, Hayllar K, Calne R, Tan KC *et al*. Influence of human leukocyte antigen matching on liver allograft survival and rejection: "the dualistic effect." *Hepatology* 1993;**17**(6):1008–1015
5. Hong G, Yi NJ, Suh SW, Yoo T, Kim H, Park MS *et al*. Preoperative selective desensitization of live donor liver transplant recipients considering the degree of T lymphocyte cross-match titer, model for end-stage liver disease score, and graft liver volume. *J Korean Med Sci* 2014;**29**(5):640–647
6. van Halteren AGS, Jankowska-Gan E, Joosten A, Blokland E, Pool J, Brand A *et al*. Naturally acquired tolerance and sensitization to minor histocompatibility antigens in healthy family members. *Blood* 2009;**114**(11):2263–2272
7. Aoki T, Sugawara Y, Takahashi M, Kawaguchi Y, Kaneko J, Yamashiki N *et al*. Living donor liver transplantation using sensitized lymphocytotoxic crossmatch positive graft. *J Gastroenterol* 2012;**47**(4):486–488
8. Hori T, Uemoto S, Takada Y, Oike F, Ogura Y, Ogawa K *et al*. Does a positive lymphocyte cross-match contraindicate living-donor liver transplantation? *Surgery* 2010;**147**(6):840–844
9. Suh K-S, Kim SB, Chang S-H, Kim SH, Minn KW, Park MH *et al*. Significance of positive cytotoxic cross-match in adult-to-adult living donor liver transplantation using small graft volume. *Liver Transplant* 2002;**8**(12):1109–1113
10. Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: history and mechanism of action. *Am J Transplant* 2006;**6**(5p1): 859–866
11. Maloney D, Liles T, Czerwinski D, Waldichuk C, Rosenberg J, Grillo-Lopez A *et al*. Phase I clinical trial using escalating

single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. *Blood* 1994;**84**(8):2457–2466

12. Book BK, Agarwal A, Milgrom AB, Bearden CM, Sidner RA, Higgins NG *et al*. New crossmatch technique eliminates interference by humanized and chimeric monoclonal antibodies. *Transplant Proc* 2005;**37**(2):640–642
13. Bearden CM, Agarwal A, Book BK, Sidner RA, Gebel HM, Bray RA *et al*. Pronase treatment facilitates alloantibody flow cytometric and cytotoxic crossmatching in the presence of rituximab. *Hum Immunol* 2004;**65**(8):803–809
14. Ishizuka T, Ishida H, Hurusawa M, Ono M, Yasuo M, Tanabe K. Improvement of cytometric detection of anti-HLA antibody using pronase in patients treated with rituximab. *Jpn J Transplant* 2007;**42**(2):143–149
15. Ishizuka T. HLA Antibody detection in organ transplantation. *Major Histocompatibility Complex* 2013;**20**(2):121–129
16. Bearden CM, Book BK, Sidner RA, Pescovitz MD. Removal of therapeutic anti-lymphocyte antibodies from human sera prior to anti-human leukocyte antibody testing. *J Immunol Methods*. 2005;**300**(1–2):192–199