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Brief communication

Changes in anti-HLA antibody titers more than 1 year after desensitization therapy with rituximab in living-donor kidney transplantation

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ABSTRACT

One hundred sixty-five kidney transplantations were performed from January 2005 to December 2007 at our hospital. Low-dose rituximab was administered to 78 patients as an induction immunosuppressant. Of the 78, 48 were donor-specific anti-HLA antibodies (DSA)-positive, and the changes in the anti-HLA antibody titers could be followed up postoperatively in 35 of these patients. Anti-HLA antibodies belonging to HLA class 1 and HLA class 2 were depleted by 74% and 86%, respectively, and remained depleted for more than 2 years. Although there were no cases of graft loss, one patient suffered from chronic AMR. Thus, we could control DSA for at least a few years after kidney transplantation using rituximab as desensitization therapy.

1. Introduction

Rituximab eliminates B-cells by complement-dependent cytotoxicity and antibody-dependent cellular toxicity. In patients undergoing kidney transplantation, rituximab has seen used in several settings: (a) treatment of post-transplant lymphoproliferative disease (PTLD); (b) ABO-incompatible transplantation; (c) treatment of antibody-mediated rejection (AMR); and (d) desensitization in HLA-sensitized patients. Since 2004, rituximab has been administered as an induction immunosuppressant for desensitization against anti-ABO antibodies and anti-HLA antibodies in recipients of living related kidney transplantation at our institution. Although there are some reports of investigation of changes in the anti-HLA antibody titers measured pre- and post-kidney transplantation, the follow-up period was not sufficiently long in any of the studies [1]. We analyzed the changes in the anti-HLA antibody titers for more than 1 year after kidney transplantation in patients with anti-donor-specific HLA antibodies before transplantation.

2. Materials and method

All study procedures were approved by the Ethics Committee of Tokyo Women's Medical University. Written informed consent was obtained from all of the study participants from our kidney transplant program. Clinical and laboratory data were extracted from electronic databases and patient medical records. The study was performed in accordance with the principles of the Helsinki Declaration.

2.1. Patients

Between April 2005 and December 2007, 165 kidney transplantations were performed at our department. Of these, 48 transplant recipients had donor-specific anti-HLA antibodies (DSA) prior to the transplant surgery; pre-transplant cytotoxicity and T-cell and B-cell FCMXs were negative in all the patients. We investigated the decrease in the titers of anti-HLA antibodies until three years after transplantation. Of the 48, the changes in the anti-HLA antibody titers with time after the surgery could be followed up in 35 patients. The anti-HLA antibody measurement was conducted by the single antigen Luminex bead assay method (LABScreen™ 100 Luminex system, One Lambda Inc., Canoga, Park, CA). We also performed quantitative analysis using MESF (Molecules of Equivalent Soluble Fluorochrome) in 30 out of all the 35 patients in this study. To convert the mean fluorescence intensity (MFI) values into MESF values, we used standard fluorescence-labeled beads with different linker lengths, obtained from Bangs Laboratories, Inc., (Fisher, IN). The Quantum TM PE MESF kit (827A) is composed of a set of five populations of calibrated fluorescent standards (500–50,000 MESF range): four populations of microbeads having different phycoerythrin (PE) fluorescence intensity values and one Certified Blank TM population. The MESF standard beads of the MESF kit were tested using the FACS machine. Reaction strength data were obtained as the MFI values. Each test was performed in triplicate. The data were converted to MESF values by extrapolating from a standard curve. To generate the standard curve,

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results ranging from 0 to 50,000 were plotted (MFI vs. MESF) using the linear regression analysis software Statview (SAS institute, Cary, NC).

2.2. Desensitization protocol and immunosuppressant treatment

Rituximab was administered at a single dose (200 mg or 500 mg) within 7 days prior to the transplantation. In addition, 3 or 4 sessions of double-filtration plasmapheresis (DFPP) were undertaken within 7 days prior to the transplantation, in order to remove anti-HLA antibodies. The immunosuppressive protocol consisted of tacrolimus, mycophenolate mofetil (MMF), methylprednisolone and basiliximab. Tacrolimus was started at the initial dose of 0.1 mg/kg, administered twice a day, and the dosage was titrated with the aim of maintaining the trough level at 10 ng/ml during the first month, 5 to 10 ng/ml during the second month, and 5 ng/ml thereafter. MMF was given at an initial dose of 2 g twice a day, which was reduced to 1.5 g on postoperative day (POD) 14. Basiliximab was administered intravenously at the dose of 20 mg before perfusion of the graft renal artery and the same dose on POD 4. Methylprednisolone was administered intravenously at the dose of 500 mg before perfusion of the graft renal artery, and subsequently, at the dose of 250 mg on POD 1, 125 mg on POD 2, 80 mg on POD 3, 60 mg on POD 4, and 40 mg on POD 5. The steroid was switched to oral methylprednisolone administered at the dose of 20 mg on PODs 6–11, 16 mg on PODs 12–17, 12 mg on PODs 18–23, 8 mg on PODs 24–29, and 4 mg thereafter.

2.3. Diagnosis of rejection

Protocol biopsy after the operation was performed at approximately 14 days and 6 months after the transplantation. Patients with complications, peri-renal infection or bleeding tendency were excluded. Any time rejection was suspected, episode biopsy was performed. The transplant kidney biopsies were not synchronized to the time-points of the assessments for anti-HLA antibody after the operation. Although the phrase “current biopsy” (in the table) is used to refer to the latest biopsy, episode biopsy was performed at the time of detection of the reappearance or de novo detection of DSA.

The type of rejection was classified according to the Banff 07 criteria. Two or three core biopsy samples were obtained using a spring-loaded 16-gauge Biopty gun under ultrasound guidance. The diagnosis of rejection was made by the same pathologist in all cases.

3. Results

3.1. Patient characteristics

The desensitization status for anti-HLA antibodies in 35 patients was assessed by the Luminex bead assay pre- and postoperatively (Table 1). Of the 35 patients, 20 were males and 15 were females. The average age was 38 years. The dose of rituximab was 200 mg in 29 patients and 500 mg in the remaining 6 patients. There were 10 patients with ABO incompatibility and 25 patients with ABO compatibility.

3.2. Desensitization of anti-HLA antibody

The postoperative mean serum creatinine level was 1.22 ± 0.35 mg/dl and none of the patients showed graft loss. Totally, among 35 patients with donor-specific anti-HLA

Table 1
Desensitization of anti-HLA antibody.

Patient initial	Age(gender)	Date of transplant	ABO	Pre-ope (class 1/class 2)	1y	2y	3y	Post-ope S-Cr (mg/dl)	Current biopsy
C.I.	60(F)	2005.8.1	Incompatible	NDSA/DSA	no/DSA	ND	no/no	0.93	No rejection
M.M.	51(M)	2005.5.27	Incompatible	DSA/DSA	no/no	ND	no/no	1.16	No rejection
N.S.	51(M)	2005.10.25	Incompatible	NDSA/DSA	no/no	no/no	no/no	1.85	No rejection
M.S.	29(F)	2005.11.1	Incompatible	NDSA/DSA	NDSA/no	ND	no/no	1.17	No rejection
H.K.	47(M)	2005.1.11	Incompatible	DSA/NDSA	NDSA/NDSA	NDSA/NDSA	NDSA/NDSA	1.15	No rejection
Y.H.	49(F)	2005.7.22	Compatible	DSA/no	no/no	no/no	no/no	0.87	No rejection
H.Y.	33(F)	2005.8.16	Compatible	NDSA/DSA	no/no	no/no	no/no	1.07	No rejection
A.T.	43(M)	2005.8.26	Compatible	NDSA/DSA	NDSA/NDSA	no/NDSA	no/no	1.56	1B,2A
Y.H.	38(M)	2005.8.30	Compatible	NDSA/DSA	no/N DSA	no/no	no/no	1.43	No rejection
N.O.	22(M)	2005.9.6	Compatible	NDSA/DSA	NDSA/no	ND	no/no	1.15	No rejection
A.K.	25(M)	2005.9.20	Compatible	DSA/no	no/no	no/no	DSA/no	1.25	CAMR
E.Y.	26(F)	2005.11.8	Compatible	DSA/no	no/no	no/no	no/no	0.92	No rejection
T.K.	58(M)	2006.1.10	Compatible	DSA/DSA	no/DSA	no/NDSA	no/N DSA	1.3	No rejection
M.K.	26(F)	2006.1.17	Compatible	NDSA/DSA	no/DSA	no/no	no/no	0.81	No rejection
C.O.	55(F)	2005.12.9	Compatible	DSA/no	no/DSA	no/no	no/no	0.87	No rejection
K.U.	39(M)	2006.1.31	Compatible	DSA/DSA	NDSA/NDSA	no/no	no/no	1.28	No rejection
Y.M.	37(F)	2006.4.11	Compatible	NDSA/NDSA	no/no	no/no	no/no	1.22	No rejection
K.T.	19(M)	2006.5.30	Compatible	DSA/no	NDSA/DSA	NDSA/DSA		2.13	AMR
M.T.	22(F)	2006.6.20	Incompatible	NDSA/DSA	NDSA/NDSA	NDSA/NDSA		1.15	No rejection
T.H.	58(F)	2006.10.3	Compatible	NDSA/DSA	NDSA/no	NDSA/no		0.87	No rejection
S.M.	50(M)	2006.12.26	Incompatible	NDSA/DSA	NDSA/NDSA	NDSA/NDSA	NDSA/NDSA	1.18	No rejection
I.Y.	50(M)	2007.2.9	Incompatible	NDSA/DSA	no/no	no/no		0.68	No rejection
K.K.	19(F)	2007.2.13	Compatible	NDSA/DSA	no/no	no/no		1.26	No rejection
T.T.	26(M)	2007.2.23	Compatible	NDSA/DSA	NDSA/no	NDSA/no		0.8	No rejection
N.H.	42(F)	2007.3.6	Compatible	NDSA/DSA	no/no	no/no		0.8	No rejection
H.I.	35(M)	2007.3.20	Compatible	NDSA/DSA	ND	no/no		1.45	No rejection
K.S.	28(M)	2007.4.10	Compatible	DSA/NDSA	NDSA/no	NDSA/no		1.16	No rejection
M.N.	42(F)	2007.4.13	Compatible	NDSA/DSA	ND	no/no		0.91	No rejection
T.U.	61(M)	2007.5.25	Compatible	DSA/no	NDSA/DSA			1.34	No rejection
K.M.	28(F)	2007.5.29	Incompatible	NDSA/DSA	ND	no/no		1.07	No rejection
H.Y.	27(M)	2007.6.19	Compatible	DSA/no	ND	no/no		1.99	No rejection
R.N.	34(F)	2007.7.3	Compatible	DSA/DSA	ND	no/no		1.51	No rejection
H.O.	43(M)	2007.9.21	Incompatible	DSA/NDSA	no/NDSA	no/NDSA		1.27	No rejection
M.H.	36(M)	2007.11.20	Compatible	NDSA/DSA	no/no	no/no		1.24	No rejection
K.S.	34(M)	2007.12.18	Compatible	NDSA/DSA	NDSA/no	no/no		1.99	No rejection

NDSA: non-donor-specific antibody.
DSA: donor-specific antibody.
no: no existance of anti-HLA antibody.
ND: not done.