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Original Article

Continuous Monitoring of Donor Specific Anti-HLA Antibody in Kidney Transplantation Patients

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Background: A positive reaction at flow cytometry crossmatch (FCXM) has been highlighted by its predictive value for clinical outcome in kidney transplantation after accumulation of large clinical data. The detection of de novo development of anti-HLA antibodies after transplantation is associated with increased rejection and decreased graft survival. In this study, we report the experience for the detection of anti-donor specific antibody (DSA) by more sensitive FCXM methods in renal transplantation patients.

Methods: T and B cell FCXMs were performed on 11 pretransplant and 51 posttransplant sera from 11 patients who received renal grafts between 2004 and 2005. The posttransplant sera were collected in specific and regular intervals from posttranspant 1 week to 1 year.

Results: Among 62 sera, four (7.8%) from 2 patients showed positive FCXM. In one patient, pretransplant serum which was negative at previous CDCXM, and 2 consecutive sera collected at 1 week and 1 month after transplantation were positive at FCXM. And the antibody identified was B51 which was specific for one of donor alleles (DSA). In another patient, FCXM became positive 1 week after transplantation although pretransplant serum had negative results at both CDCXM and FCXM. Both patients had experienced more than one rejection episodes.

Conclusions: Detection of DSA with more sensitive technique such as flow cytometry based method clearly displayed a beneficial effect for prediction of clinical outcome as a part of pretransplant compatibility test, and also as a posttransplant monitoring test to identify the de novo production of clinically significant DSA.

Key Words: Kidney transplantation, Histocompatibility antigens (HLA), Donor specific antibody, Flow cytometry crossmatch
중심 단어: 신장이식, 조직적합성항원, 공여자특이항체, 유세포분석교차시험

Introduction

The reactivity between HLA antigen of donor and anti-HLA antibody of recipient in pretransplant assessment is one of the critical factors to determining successful transplantation, especially in renal transplantation.(1) The detection of preformed antibody against donor HLA antigen has been widely performed with cross match test (XM) using complement dependent cytotoxicity (CDC).(2) However, in cases where the presence of low level of anti-HLA antibodies or of few memory cells, the conventional CDC method (CDCXM) does have limited sensitivity for reflecting sensitization status of the recipient accurately. Flow cytometry had been introduced for HLA XM during the mid-1990s and is currently known to be up to 5-25 times more sensitive than CDC method.(1,3-5) Although there have been debates about the clinical significance of positive flow cytometric XM (FCXM) which suggest the presence of low titer and/or non-complement fixing antibodies,(6,7) there are many clinical reports showing significant impact of positive FCXM even in the absence of CDCXM positive antibodies.(4,8,9) Gebel et al. have reported the increased incidences of early graft rejection and primary graft failure, and reduced rate of graft survival in patients with pretransplant CDCXM negative, but with FCXM positive.(10) Posttransplant presence of DSA, due to restimulation of previously sensitized memory population to donor antigens or due to formation of de novo DSA, has been reported to increase the risk for acute and chronic rejection and decrease allograft survival.(11-16) FCXM has clear advantages

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compared to CDCXM in terms of sensitivity and laboratory practicability for simultaneous evaluation of T and B lymphocytes; it allows less subjective reading of results and repeated measurement of DSA, although it needs more or less to be measured by technical experts and its results interpreted by clinical experts.(17)

In this study, we have measured the presence of pretransplant DSA in kidney transplant patients by CDCXM and FCXM and have continuously monitored the posttransplant DSA up to 1 year by FCXM. The results have been correlated with patients' clinical status to investigate the clinical significance of monitoring the presence of DSA pre- and post- renal transplantation.

Materials and Methods

1) Study Design and Study Population

From January 2004 to January 2006, 11 patient and donor pairs for renal transplantation were enrolled to be monitored for the presence and/or the formation of donor specific anti-HLA antibody (DSA) prospectively during 1 year posttransplant at Ewha Womans University Mokdong Hospital. The pretransplant crossmatches were performed with CDC microlymphocytotoxicity test including AHG phase (AHG CDCXM). Retrospectively, the pretransplnat sera and the posttransplant sera collected at 1 week, 1, 3, 6 and 12 months after renal transplantation were subjected to FCXM with freshly collected donor lymphocytes at 6 and/or 12 months to test the presence of DSA. The protocol was approved by hospital internal review board and informed consents were acquired from patients and donors before starting the study. The characteristics of patients and donors are summarized in Table 1. The mean age of recipients was 40±7 (23-48), excluding one 11 year old patient who was diagnosed as IgA nephropathy, and the male to female ratio was 1 : 1.2. Most patients were diagnosed with chronic renal failure 1 to 5 years ago and were undergoing continued dialysis. Among 11 renal transplantation patients, five received the renal graft from related donors and 6 received from unrelated donors. They were all matched for ABO blood group except one patient with B to AB minor mismatch. The degree of HLA mismatch was 3-6 loci, not including one patient who had received a graft from an HLA matched sibling. The transplantation across genders was in 7 patients. Five patients who had histories of multiple pregnancies received grafts from husbands or son. None of the patients received preconditioning therapy. Mean follow up duration was 33±11 (12-46) months. The increase of creatinine (over 1.4 mg/dL) without evi-

Table 1. Demography and clinical characteristics of patients

Case No.	Recipients				Donors			No. of HLA mismatches	Baseline Immunosuppression
	Age	Gender	Etiology of renal disease	Pretransplant sensitization episode	Type of transplant	Age	Gender		
1	39	F	Unknown	P, O	Unrelated (Sp)	41	M	4	CMP
2	40	F	Unknown	P	Unrelated (Sp)	48	M	5	CMP
3	40	M	Hypertension	P	Unrelated (Sp)	37	F	4	MP+Tacrolimus
4	47	M	Hypertension	-	Unrelated (R)	35	M	6	CMP
5	39	F	Hypertension	P	Unrelated (Sp)	40	M	4	CMP
6	39	F	IgA Nephropathy	P, T	Related (Si)	34	M	0	CMP
7	38	F	Hypertension	P	Related (F)	62	M	3	CMP
8	11	M	Alport syndrome	-	Related (F)	40	M	3	CMP
9	23	M	Hypertension	-	Related (Si)	26	M	3	CMP
10	47	F	Single kidney, Hypertension	P	Related (So)	20	M	3	CP
11	48	M	Unknown	-	Unrelated (W)	37	F	3	CMP

Abbreviations: P, pregnancy; O, operation (without known transfusion history); T, RBC transfusion; Sp, spouse; R, relative; Si, sibling; F, father; So, son; W, wife; CMP, cyclosporine; mycophenolate; prednisolone; MP, mycophenolate; prednisolone; CP, cyclosporine; prednisolone.

dence of infection or drug toxicity but responding steroid treatment was defined as an acute rejection episode. In case of performing kidney biopsy, cell mediated rejection was diagnosed based on Banff 97 classification.

2) HLA Typing

HLA Class I was typed with microlymphocytotoxicity method using HLA typing Terasaki plate (One Lambda, USA) and HLA Class II was typed with PCR-SSOP method using 2 pairs of allele specific primers for DRB1 locus and probe hybridization reaction (InnoLipa, USA).

3) Complement Dependent Cytotoxicity Crossmatch (CDCXM)

Each of the donor's T and B cells separated by nylon wool column were incubated with the patient's serum, and then anti-human kappa antibody, for improving sensitivity of DSA detection, and complement were added sequentially. Positive reaction was defined as presence of more than 10% of dead cells compared to negative control by dye exclusion method using eosin Y.

4) Flow Cytometry Crossmatch (FCXM)

The patient's serum was incubated with 0.5×10^6 donor lymphocytes for 30 minutes at room temperature. After washing, cells were reincubated with 20 μL of 1 : 10 diluted FITC-F(ab')2 anti-human IgG (Beckman Coulter, USA) and 10 μL of anti-CD3-PE and anti-CD19-PE (Becton Dickson Bioscience, USA) for T cell- FCXM and B cell-FCXM, respectively. Cells were analyzed with FC500 flow cytometry (Coulter Diagnostics, CA, USA). Mean channel shift (MCS) has been calculated by subtracted patient's mean fluorescence intensity (MFI) with negative control's and MCS over 5 for T cells, and over 10 for B cells have been interpreted as presence of DSA in patient's serum. In any case, the percent displacement of positive population more than 10% of negative cutoff also has been interpreted as presence of DSA. Negative control serum was the pooled serum of 5 healthy males with AB blood group and without history of allo-sensitization. The negative cutoff was determined from the fluorescence intensity of 20 healthy individuals tested with negative control sera. The patient's autocontrol has been included to exclude the presence of autoantibodies.

5) Identification of Specificities of Anti-HLA Antibodies

Once the sera showed positive results with FCXM or CDCXM, the specificities were determined with ELISA based Lamda Antigen Tray (LAT^{TM}, One Lambda, CA, USA). The patient's serum was incubated with 28 HLA Class I and 12 Class II extracted antigens coating microplate and then alkaline phosphatase conjugated anti-human IgG was added to develop the color changes in wells with specific antigen and antibody reactions.

Results

1) Pre- and Post-transplant Detection of Donor-specific Antibody (DSA)

All of the patients in this study had negative results for pretransplant CDCXM including AHG phase, and any patients with positive pretransplant CDCXM were excluded. Total of 11 pretransplant and 51 posttransplant sera from 11 patients collected at specified time points for 1 year after renal transplantation were tested for the presence of DSA by FCXM retrospectively. Among 11 pretransplant sera, one (9.1%) showed a positive result at FCXM although prior CDCXM was negative. Among 51 post-transplant sera, three serum samples (5.9%) from 2 patients (P3 and P5) (18.1%) were positive for DSA by FCXM at post-transplant 1 week, and at post-transplant 1 week and 1 month, respectively.(Table 2) P3 who was negative for both CDCXM and FCXM in pretransplant serum became to have positive for T cell FCXM at post-transplant 1 week, but became undetectable at 1 month. P5 who had positive for T and B cell FCXM, but not for CDCXM in pre-transplant serum, significantly elevated level of DSA at post-transplant 1 week. The DSA was persisted until 1 month but not detected at 3 months after transplantation. The specificities of DSA tested by ELISA-based PRA identification method at pre- and post-transplant sera of P5 were identified as anti-B51, which was specific for one of HLA-B alleles of the donor. In P3, the specificity of antibody detected by

Table 2. Immunologic characteristics and clinical outcomes of renal transplant patients

Case No.	HLA mismatches			Pretransplant XM		Posttransplant XM	Specificity of Antibodies	Rejection episode	Tissue biopsy	Follow-up period (months)	Graft loss
	A	B	DRB1	AHG CDC	FC	FC					
1	1	2	1	-	-	-		-	ND	46	-
2	2	1	2	-	-	-		-	ND	44	-
3	2	1	1	-	-	+	NS*	+	ATN, AR	43	-
4	2	2	2	-	-	-		-	NR, drug toxicity	39	-
5	0	2	2	-	+	+/+†	B51‡	+	AR	37	-
6	0	0	0	-	-	-		-	ND	12	-
7	1	1	1	-	-	-		-	ND	35	-
8	1	1	1	-	-	-		-	Minimal tubulitis	34	-
9	1	1	1	-	-	-		-	ND	26	-
10	1	1	1	-	-	-		-	ND	22	-
11	1	1	1	-	-	-		-	ND	22	-

Abbreviations: AHG CDC, antihuman globulin phase CDC crossmatch; FC, flow cytometry crossmatch; ND, not done; ATN, acute tubulointestinal nephritis; AR, acute rejection; NR, no evidence of rejection. *No specificity identified by LAT; †Results of posttransplant 1 week and 1 month; ‡Specificity identified from pre- and post-transplant XM positive sera.

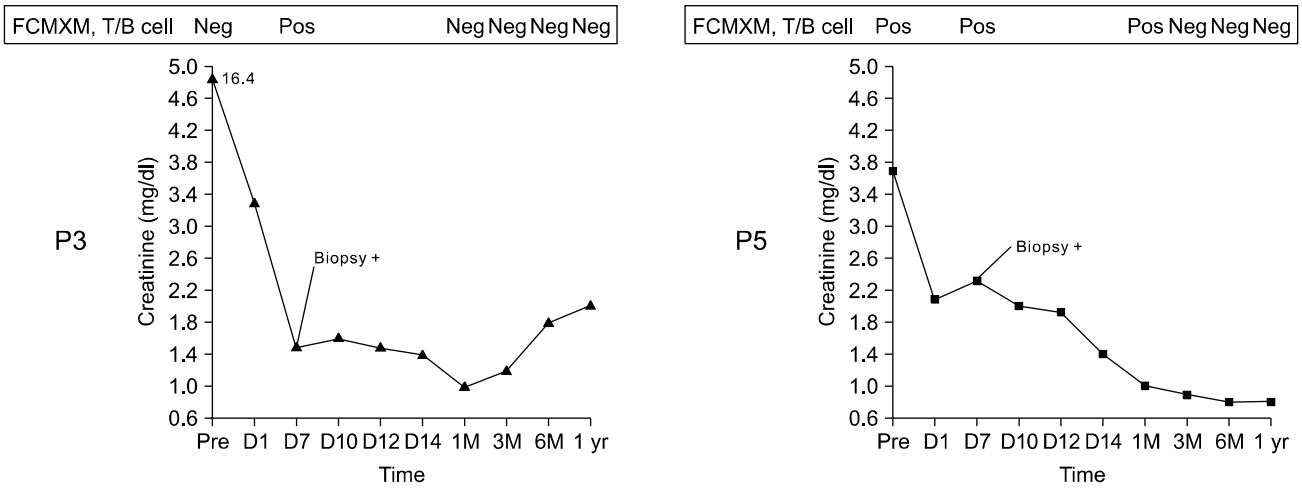

Fig. 1. Clinical and laboratory manifestations of 2 FCXM positive patients (P3 and P5) during 1 year period post renal transplantation.

FCXM was not determined with ELISA method. In all sera tested for PRA identification, anti-HLA other than DSA was not detected.

2) Clinical Courses in DSA-positive and -negative Patients

Both P3 and P5 who had DSA by FCXM had experienced biopsy proven acute rejection episodes at posttransplant day 4 and day 7, respectively (Fig. 1). During 43 and 37 months of follow up period, both patients had increased level of Cr up to 2.4 mg/dL intermittently, although Cr level at the end of follow up period were maintained at 1.5 and 0.9 mg/dL, respectively. Among the other 2 patients who had elevated Cr but negative for DSA, one (P4) was diagnosed as drug toxicity at renal biopsy and the other (P11) who was suspicious for immune rejection was not proved by renal biopsy.

Discussion

In renal transplantation, the presence of preformed antibody against donor HLA predisposes acute and subacute post-transplant rejection and closely related with poor graft survival.(1,2,18-20) Moreover, posttransplant presence of DSA after restimulation of pre-

viously sensitized memory population to donor antigens or due to formation of de novo DSA would increase the risk of acute and chronic rejection and decrease the allograft survival.(11-16) In this study, we have prospectively monitored 11 kidney transplant recipients for the presence and the formation of DSA by FCXM during 1 year after transplantation. In one patient, the antibody was detected only at 1 week post-transplant and disappeared at 1 month follow up serum; however, the other patient who already had low titer DSA undetectable by CDCXM before transplantation, the antibody titer increased and persisted until 1 month posttransplant. Two patients (18%) out of 11 developed post-transplant DSA and both had more than one episode of biopsy proven rejection in addition to elevated creatinine levels during the first year after transplantation. Among 9 patients who did not develop post-transplant DSA, none were diagnosed as having rejection episodes related with immunological events.

The frequency of post-transplant anti-HLA antibody has been reported in wide range such as 1.6-60% mainly due to variability in the type of assays used, the type of patient populations analyzed, or variable times of sample collection.(11) Scornik et al. has observed the presence of alloantibodies in 25% of recipients after renal transplantation.(22) At a prospective clinical trial conducted by an international cooperative group,(23) the overall frequency of anti-HLA antibodies was 20.9% in kidney transplant recipients. In unsensitized patients, 14.7% developed de novo anti-HLA antibodies during 1 yr of follow-up. Piazza et al. (24) prospectively screened 120 unsensitized kidney recipients for DSA at 1 yr after transplantation by FCXM and FlowPRA. Overall, 24.2% had developed DSA and most of them were detected within the first 3 months after transplantation. They have shown that the patients with DSA had higher incidence of acute rejection episodes, more allograft failure and higher creatinine levels at 2 yr after transplantation compared with patients without DSA. Among Korean, Kim et al. (25) has reported 5.2% of patients converted PRA test to positive after kidney transplantation, and 29% of patients among them who persistently positive for PRA test had higher incidence of acute rejection episodes. Considering the facts that both patients had developed or increased titers of DSAs within a week after transplantation in this study and that Piazza et al. had observed DSA mostly within 3 months of transplantation, it would be useful to perform tests for the detection of de novo DSA or of increased titer during early phase of transplantation. In one patient, we could not determine the specificity of antibody although FCXM was positive and tissue biopsy finding was compatible with acute rejection. In this case, the positive FCXM might be due to very low titer of DSA below the sensitivity of ELISA based antibody identification or antibodies against non-HLA antigens which can trigger the immune rejection. We did not perform PRA test for all recipients, so we could not exclude the possibility that a few patients might have non-DSA which did not provoke immunological reaction to donor. But in two patients who experienced acute rejection with positive FCXM, non-DSA antibody was not identified at PRA identification test.

For the detection of anti-HLA antibody, more sensitive methods than conventional CDC method such as ELISA-, flow cytometry- or luminex bead- based methods have been introduced.(26) The fact that one patient who experienced rejection episode had positive FCXM but not CDCXM at pretransplant test shows that higher sensitivity is more predictive of immunological event. Most of these methods have not only superior sensitivity but also technical advantage to perform routinely at clinical laboratories compared to conventional CDC method. Especially, detection of DSA showed substantial benefit for the prediction of post-transplantation rejection episode than detection of non-DSA, although for the detection of DSA, prior preparation of or preservation of donor proteins or cells, and standardization effort are needed.(27)

In this report, we are showing that monitoring of presence of DSA at posttransplantation stages is necessary to predict the unwanted immunological events, such as rejection, and to customize the immunosuppressive strategies for enhanced clinical outcome. For this, more sensitive laboratory methods based on flow cytometry or luminex bead assay would be needed.

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