Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Significance of qualitative and quantitative evaluations of anti-HLA antibodies in kidney transplantation

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Keywords
anti-HLA antibody, donor-specific antibody, kidney transplantation, MESF, quantitative analysis.

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Received: 9 May 2010
Revision requested: 27 June 2010
Accepted: 16 August 2010
Published online: 8 September 2010

doi:10.1111/j.1432-2277.2010.01166.x

Summary

In this study, we retrospectively investigated the relationship between the presence/titers of donor-specific (DSA)/nondonor-specific antibody (NDSA) and the rate of graft rejection after transplantation. The subjects comprised 34 recipients who tested positive by FlowPRA® Screening. The recipients were divided into two groups; 22 recipients with DSA and 12 recipients with NDSA, as detected using FlowPRA® Single Antigen I and II beads. The antibodies were also quantitatively examined using the molecules of equivalent soluble fluorochrome (MESF) method. Nine of the 22 recipients with DSA (9/22, 40%) developed antibody-mediated rejection (AMR), while none of the 12 recipients with NDSA (0/12, 0%) developed AMR ($P < 0.01$). In a quantitative analysis of the MESF data, patients with DSA with MESF values of over 3000 frequently showed AMR (8/11, 73%). In contrast, one of the patients with DSA with MESF values of <3000 showed AMR (1/11, 9%). One of the 12 patients (1/12, 8%) with NDSA showed cellular rejection (T-cell-mediated rejection), regardless of the MESF values. In patients with DSA, an MESF value of 3000 may be a useful cutoff value for identifying patients at a high risk for AMR.

Introduction

Antibody-mediated rejection (AMR) is recognized as a distinct entity from cellular rejection. AMR occurs as a result of antibody deposition on the graft endothelium and subsequent complement activation. Histologic and immunologic studies on renal graft biopsies obtained from patients with AMR have revealed intravascular macrophages, and immunoglobulin and complement deposition in the capillaries [1–3]. AMR is triggered by humoral immunity mediated by various kinds of antibodies, including anti-human leukocyte antigen (HLA) antibodies produced by the plasma cells in sensitized recipients. HLA-specific alloantibodies found in post-transplant patients have been shown to be strongly associated with poor graft function and graft loss [4].

With the development of more sensitive assays involving flow-cytometric analysis, it has now become clear that the titers of DSA vary widely among patients and that the serum DSA titers may be the major determinant of allograft injury [4]. However, lack of standardization of flow-cytometric methods around the world has made it difficult to evaluate precisely the humoral immunologic status before and after transplantation [5–8]. Currently, the development of FlowPRA® Single Antigen I and II beads and LABScreen Single Antigen multiplex solid phase immunoassay has made it possible to better identify donor-specific (DSA) or nondonor-specific (NDSA) anti-HLA antibodies qualitatively. Moreover, the development of the molecules of equivalent soluble fluorochrome (MESF) method, first reported by Schwartz, *et al.* [9], has also made it possible to better analyze anti-HLA antibody titers quantitatively. In this study, we investigated the relationship between the presence/titers of DSA/NDSA and the rate of graft rejection after transplantation.

Materials and methods

Patients and immunosuppressive regimens

We performed 172 living related kidney transplantations between 2003 and 2005 at our institution. All candidates for transplantation at our institution receive FlowPRA® Screening (FlowPRA®; One Lambda, Canoga Park, CA, USA) for determination of immunosuppressive regimens. The subjects of this study were 34 recipients who tested positive on FlowPRA® Screening before the transplantation. The recipients were divided into two groups; 22 recipients with DSA and 12 recipients with NDSA, as detected using Flow PRA® Single Antigen I and II beads (One Lambda). The frozen serum samples used in this study were kept until thawing for analysis in a refrigerator at −90 °C. All the recipients enrolled in this study received similar triple therapy for immunosuppression, consisting of oral tacrolimus (FK), mycophenolate mofetil (MMF) and methylprednisolone (MP). FK and MMF were used as the alternative calcineurin inhibitor and antimetabolite immunosuppressive agent to cyclosporine (CsA) and azathioprine, respectively. FK (Prograf®; Astellas Fujisawa, Osaka, Japan) was started 7 days before the transplantation at the dose of 0.1 mg/kg/day, and the dose was adjusted to maintain a trough level of FK in whole blood between 8 and 12 ng/ml during the first month postoperatively, between 7 and 9 ng/ml during 2–3 months after the transplantation, and between 4 and 6 ng/ml thereafter. MMF (Cellcept®; Roche, Nutley, NJ, USA) was also started 7 days before the transplantation at the dose of 2000 mg/day, with the dose decreased to 1000–1500 mg/day during the first month postoperatively, depending on the count of white blood cells. MMF is given in reduced doses in some recipients. However, in our institution, higher incidence rate of adverse events like newly developed antibody production cannot be significantly observed in MMF-reduced dose group.

Methylprednisolone was administered intravenously at doses of 500, 250 and 125 mg/day on the day of transplantation, day 1 after the operation and day 2 after the operation respectively. Oral MP was started on day 3 after the operation at the dose of 80 mg/day, and the dose was then tapered to 6–8 mg/day within 1–2 months after the transplantation. The immunosuppressive protocol for patients with positive PRA (plasma renin activity) screening results has been described in greater detail in our previous study report [10,11]. In brief, in addition to the conventional triple immunosuppressive regimen, three or four sessions of double-filtration plasmapheresis (DFPP) were undertaken to remove the antibodies prior to transplantation. Moreover, basilliximab, the anti-CD25 antibody, was also given on day 0 and day 4 after transplantation at the dose of 20 mg on each of the days, as a desensitization protocol, in all the recipients enrolled in this study (Fig. 1).

Sera were separated from the blood samples obtained from the recipients and donors after obtaining their informed consent.

Graft biopsies

Biopsy was carried out 2–3 times, using a 16-gauge needle. Postoperative protocol biopsies (at 0 h, <6 months and more than 6 months), as well as episode biopsies, were obtained from all the recipients. Among the 22 recipients who developed DSA within 6 months of operation, 14 recipients (14/22, 64%) underwent episode biopsies because they had clinical symptoms, such as a decrease in the urine volume, poor blood flow (diastolic pressure) in the graft shown by ultrasonography,

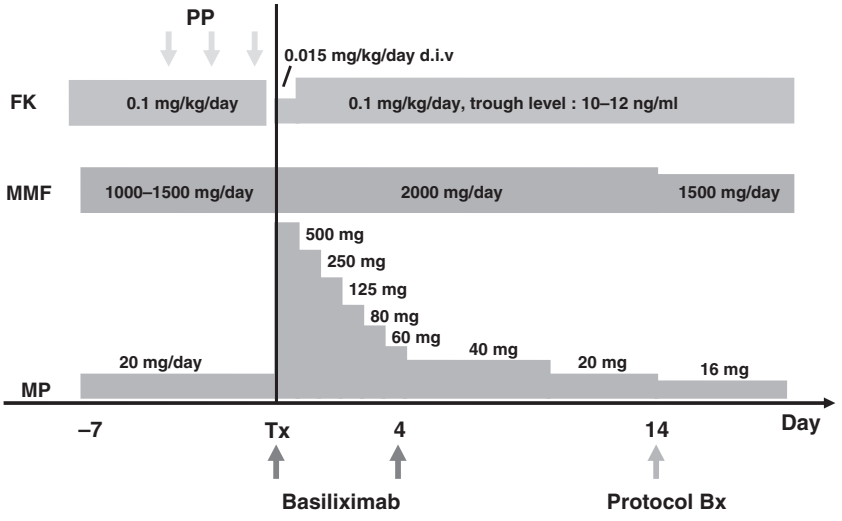

Figure 1 Immunosuppressive regimen for patients positive for PRA (plasma renin activity) (2003–2005). FK, tacrolimus; MMF, mycophenolate mofetil; MP, methylprednisolone.

moderate to high-grade fever, a decrease in platelet and LDH elevation, etc., suggestive of AMR. In contrast, among the 12 recipients who developed NDSA within 6 months of operation, only three recipients (3/12, 25%) underwent episode biopsies. In addition to these recipients, protocol biopsies were routinely performed in all the other recipients before 6 months. After 6 months, all the recipients underwent protocol biopsies even if they did not have any clinical symptoms, regardless of whether they had DSA or NDSA. When a graft biopsy confirmed AMR, the recipients were treated with MP pulse therapy and DFPP until the recovery of the graft's function. OKT3 was also given to three recipients with DSA. All the grafts were clinically rescued after these treatments. Once the graft function had recovered, the improvement in the pathologic findings was not confirmed by a second biopsy.

All biopsies were evaluated by light microscopy and immunofluorescence staining for C4d. Briefly, the specimens were fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin and cut into 2-μm sections. The sections were stained with hematoxylin and eosin, periodic acid-Schiff, Masson trichrome and periodic acid methenamine silver stains for light microscopy. For immunohistochemistry, the paraffin sections on glass slides coated with saline were stained using a peroxidase-labeled streptavidin–biotin staining kit (DAKO, Carpinteria, CA, USA). The primary antibodies used were rabbit polyclonal antibodies against immunoglobulin G (IgG), immunoglobulin A and C3 and immunoglobulin M (IgM) (Hoechst, Behringwerke, AG, Maburg, Germany). The pathologic findings were classified according to the Banff 2007 working classification and the Banff 2005 Update Edition, and comparatively evaluated in the recipients.

Briefly, AMR was defined as (i) C4d and/or (rarely) immunoglobulin deposition in peritubular capillaries; (ii) serologic evidence of circulating antibodies to donor HLA, etc.; and (iii) morphologic evidence of acute tissue injuries. Chronic AMR (CAMR) was also defined using the Banff criteria.

Only one pathologist made the diagnosis on examined graft specimens at our institution.

Determination of anti-HLA antibodies (FlowPRA® Screening and FlowPRA® Single Antigen I and II beads)

The serum was allowed to react with 10 mM of dithiothreitol at 37 °C for 10 min to eliminate immunoglobulin M (IgM). The disappearance of IgM from the serum was confirmed using rabbit anti-human IgM antibody (DAKO, Osaka, Japan) as the secondary antibody, before conducting the analysis using ordinary FlowPRA® Screening. Beads coated with the HLA antigens were added to the patient's serum using the FlowPRA® Screening, and left at room temperature for 30 min. Then, the second antibody, PE-labeled anti-human IgG antibody was added, and the reaction mixture was allowed to stand for another 30 min. After washing twice, determination was carried out using fluorescence-activated cell sorting (FACS), according to the manufacturer's instructions (Becton Dickinson, Los Angeles, CA, USA). Sera with HLA antibodies detected by the FlowPRA® Screening were also tested by FlowPRA® Single Antigen I and II beads, as reported previously. PCR-SSP technology (low and high resolution Olerup SSP Geno Vision VertriebsmbH kits, Vienna, Austria) was used for HLA class I & II DNA typing.

Data acquisition and conversion to MESF

To convert the mean fluorescence intensity (MFI) values into MESF values [9], we used standard beads labeled with fluorescence having different linker lengths, obtained from Bangs Laboratories, Inc. (Fisher, IN, USA). The Quantum™ PE MESF kit (827A) (Bangs Laboratories, San Francisco, CA, USA) 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™ 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 a standard curve, the results ranging from 0 to 50 000 were plotted (MFI versus MESF) using the linear regression analysis software, Staview (SAS Institute, Cary, NC, USA).

As reported previously by Vaidya [12], we also established a cutoff value by comparing the MESF values and FlowPRA® Single Antigen I and II beads results for negative control sera and positive diluted sera ranging from undiluted to a dilution of 1:512. Negative and positive control sera were collected from 20 normal healthy volunteers and 20 highly sensitized recipients respectively. The average MESF value of the negative control using FlowPRA® Screening was 2980 ± 404. According to this result, we determined the cutoff value as 3000 units.

Statistical analysis

The significance of differences in the MESF values of various HLA-specific antibodies was calculated by Fisher's exact test. Data were expressed as the mean ± SD. Statistical comparisons between the two groups were conducted by Student's *t*-test or Mann–Whitney *U*-test. One-way analysis (ANOVA) was used for comparisons

among the three groups. $P < 0.01$ was considered as denoting significance in all the tests.

Results

Patients

The subjects in this study were 34 patients who were FlowPRA® Screening test-positive before transplantation. As shown in Table 1, all the subjects were divided into two groups according to the presence of DSA and NDSA, as detected by FlowPRA® Single Antigen I and II beads assay. DSA and NDSA usually coexist in the same patient. A patient with even one DSA was classified in the DSA group. In contrast, the NDSA group included patients with only NDSA, but not DSA.

There were no significant differences in the gender, average age, years after transplantation, history of pregnancies and blood transfusion or PRA class I values between the DSA and NDSA groups. The PRA class II values were significantly higher in the DSA group than in the NDSA group. In the DSA group, the complement-dependent cytotoxic crossmatch (CDCXM) test was positive in two cases and the flow-cytometric crossmatch (FCXM) test was positive in four cases. In contrast, in the NDSA group, the CDCXM test was positive in one case and the FCXM was positive in one case. Two cases that were both CDCXM-positive and FCXM-positive in the DSA group lost their grafts, while none of the patients in the NDSA group experienced graft loss. When the MESF values and the CDCXM/FCXM test results were compared, CDCXM-B cells were positive in cases with an MESF value of more than 150 000, while FCXM B cells were positive in cases with an MESF value of more than 16 000 (data not shown). At our institution, T-cell crossmatch test (CDCXM/FCXM) positive is not considered to be indication for kidney transplant recipients.

Table 1. Patients' characteristics.

	DSA	NDSA	*P*-value
N	22	12	
F/M	12/10	5/7	NS
Age	42 ± 10	38 ± 9	NS
Years after Tx	6.6 ± 1.2	4.9 ± 1.6	NS
1st/2nd/3rd	18/3/1	10/2/0	NS
Pregnancy	9/12	3/5	NS
Blood transfusion	11/22	5/12	NS
PRA screening (class I/II)	54.3/52.1	62.1/9.7	NS/<0.01*
Positive CDCXM/FCXM	2/4	1/1	NS
Graft loss	2	0	NS

DSA, donor-specific antibody; NDSA, nondonor-specific antibody; Tx, transplantation; PRA, plasma renin activity; CDCXM/FCXM, complement-dependent cytotoxic crossmatch/flow-cytometric crossmatch.
*No significant difference in class I, but significant difference in class II.

Comparative study of the pathologic findings and MESF values

As shown in Tables 2–4, patients with DSA with MESF values of over 3000 frequently showed AMR (8/11, 73%). Among these eight AMR recipients with DSA with MESF values over 3000, four recipients including two graft loss showed FCXM B cells-positive. Other four AMR recipients with MESF over 3000 showed negative in both FCXM and CDCXM tests. In contrast, one of the patients with DSA with MESF values of <3000 showed AMR (1/11, 9%). One of the 12 patients (1/12, 8%) with NDSA showed cellular rejection (T-cell-mediated rejection, TMR), while no evidence of any rejection was observed in the remaining 11 (11/12, 91%), regardless of the MESF values (Tables 2 and 3).

Six months post-transplant, graft biopsies revealed CAMR in all of the nine recipients with DSA who developed AMR (9/9, 100%), including the two patients with graft loss (Table 2). In contrast, none of the patients with NDSA, even those with MESF values of over 100 000, developed AMR or CAMR postoperatively (Table 3).

The patients with second and third grafts (KA and TK; see Table 2) had very high MESF values of over 200 000 because of repeated HLA mismatching with the donors. In particular, TK had been sensitized by DR15 twice by the second (grandmother; maternal mother) and third (mother) donors. Both patients experienced vigorous AMR very early after transplantation, followed by intensive anti-rejection therapies. KH, who underwent a second transplant, had NDSA DR1 with a weak MESF value (2479).

The creatinine clearance (Ccr) was also calculated in the patients, using Cockcroft's formula. As shown in Fig. 2, the average Ccr in the NDSA group was 72 ml/min, while in the DSA group, the Ccr was 40 ml/min in the patients with MESF value over 3000 and 62 ml/min in those with MESF value <3000.

MESF value of HLA specificities by FlowPRA® Single Antigen I and II beads assay

HLA-specific antibodies of sera from 22 recipients with DSA were identified using FlowPRA® Single Antigen I and II beads assay. Table 5 demonstrates MESF values of corresponding HLA antibodies shown in Table 2. HLA-specific antibodies with MESF value >3000 were considered unacceptable, although one patient at a MESF value of 2152 (A24) was unacceptable. MESF values of all class II (DR10, 9, 15) DSA were above 3000.

Table 2. Patients with DSA.

Patient (gender)	Tx (living/deceased)	DSA	MESF value	Pathologic findings		Ccr (ml/min)
				Before 6 months	After 6 months	
MESF > 3000						
HA (F)	Living	A24	13 551	No AR (1 month)	No AR (1 year)	45
MK (M)	L	DR4	3493	No AR (3 weeks)	No AR (10 months)	52
FI (M)	L	DR10	4132	TMR (3 weeks)	No AR (1 year)	40
HY (F)	L	DR10, A26	4835, 2000	AMR (1 month)	CAMR (9 months)	HD
YT (F)	L	DR9	23 760	AMR (3 weeks)	CAMR (8 months)	HD
KA (F)	L (2nd)	DR15	414 664	AMR (2 weeks)	CAMR (9 months)	54
ST (F)	L	A24, A2	3383, 3002	AMR (1 month)	CAMR (10 months)	43
TK (M)	L (3rd)	DR15	245 402	AMR (1 month)	CAMR (11 m)	34
NF (F)	L	A26	3383	AMR (3 weeks)	CAMR (7 months)	38
SR (M)	L (2nd)	B52, B51	3563, 900	AMR (10 days)	CAMR (6 months)	32
NM (F)	L	DR9	29 030	AMR (4 months)	CAMR (8 months)	38
MESF < 3000						
TH (M)	L	B35	2152	No AR (2 weeks)	No AR (1 year)	67
YS (M)	L	DR16	2481	No AR (2 weeks)	No AR (1 year)	65
AK (F)	L	A31, A2	1176, 903	No AR (3 weeks)	No AR (9 months)	69
KA (F)	L	A24	1047	No AR (1 month)	No AR (11 months	71
MY (F)	Deceased	B51, A4	1369, 1002	No AR (1 month)	No AR (1 year)	70
WM (M)	L	B52, A2	920, 808	No AR (3 weeks)	No AR (1 year)	61
TR (F)	L	B51	1759	No AR (1 month)	No AR (2 years)	62
KY (M)	L	DR16	2402	No AR (2 months)	No AR (1 year 9 months)	55
KH (M)	L (2nd)	DR1	2479	No AR (2 months)	No AR (10 months)	69
KA (F)	L	B51	1421	TMR (1 month)	Not done	45
FM (M)	L	A24	2152	AMR (1 month)	CAMR (9 months)	34

DSA, donor-specific antibody; Tx, transplantation; MESF, molecules of equivalent soluble fluorochrome; Ccr, creatinine clearance; AR, allograft rejection; AMR, antibody-mediated rejection; CAMR, chronic AMR; TMR, T-cell-mediated rejection; HD, hemodialysis.

Table 3. Patients with NDSA.

Patient (gender)	Tx (living/deceased)	NDSA	MESF value	Pathologic findings		Ccr (ml/min)
				Before 6 months	After 6 months	
AA (M)	L (living)	A2, B4	4509, 3200	No AR	No AR	68
TK (M)	L	A24, A2	110 930, 4500	No AR	No AR	70
UY (F)	L	A2, A51, B12	6780, 5490, 800	No AR	No AR	65
TT (F)	L	A26	2100	No AR	No AR	72
IK (F)	L	DR8, A2	10 900, 1020	No AR	No AR	73
OI (M)	L	A12	1050	No AR	No AR	65
TS (M)	L	A2, DR4, B34	2100, 900, 890	No AR	No AR	69
TT (F)	L	A4	6790	No AR	No AR	64
MN (M)	L	A2	1520	TMR	No AR	70
WI (F)	L (2nd)	A4, B11	121 200, 1100	No AR	No AR	69
NN (M)	L	A9	130 40	No AR	No AR	68
KH (F)	L	A1, B34,	5200, 2050	No AR	No AR	67

NDSA, nondonor-specific antibody; Tx, transplantation; MESF, molecules of equivalent soluble fluorochrome; Ccr, creatinine clearance; AR, allograft rejection; TMR, T-cell-mediated rejection.

Discussion

The development of novel immunosuppressive regimens has improved the scope of kidney transplantation, such as transplantation across blood barriers, etc. Especially, the currently used standard immunosuppressive protocol including anti-CD 20 or anti-CD25 antibody enables successful kidney transplantation even in immunologically high-risk recipients. However, we still sometimes come across difficult cases with graft loss, most of

Table 4. The relationship between MESF value and acute rejection in patients with donor-specific antibody.

MESF value (*N*)	AMR	TMR	No rejection
3000< (11)	*N* = 8 (8/11, 73%)	*N* = 1 (1/11, 9%)	*N* = 2 (2/11, 18%)
3000> (11)	*N* = 1 (1/11, 9%)	*N* = 1 (1/11, 9%)	*N* = 9 (9/11, 82%)
P-value	<0.01	NS	<0.01

MESF, molecules of equivalent soluble fluorochrome; AMR, antibody-mediated rejection; TMR, T-cell-mediated rejection.

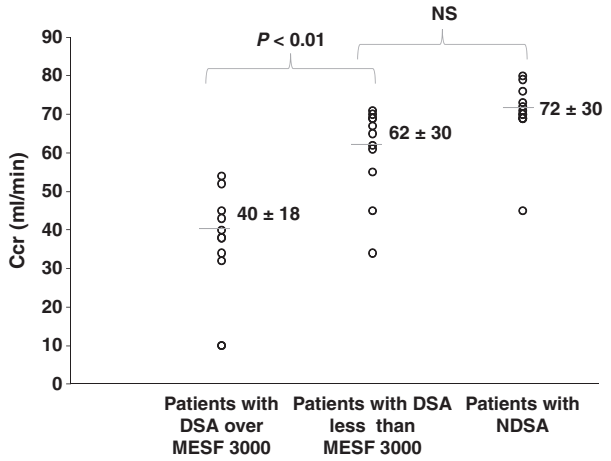

Figure 2 Graft function (creatinine clearance, Ccr). The Ccr was also calculated in the patients, using Cockcroft's formula. As shown in this figure, the average Ccr in the nondonor-specific antibody (NDSA) group was 72 ml/min, while in the donor-specific antibody (DSA) group, the Ccr was 40 ml/min in the patients with molecules of equivalent soluble fluorochrome (MESF) value over 3000 and 62 ml/min in those with MESF value <3000.

which show evidence of severe AMR in later graft biopsies.

One of the other major reasons for the high success rate of kidney transplantation is the development of advanced technologies for the detection of anti-HLA antibodies. However, not all institutions always adopt new techniques to detect anti-HLA antibodies, and progress in the use of flow-cytometric analysis has also contributed to the excellent survival rate of transplanted grafts, as well as to a better understanding of transplant immunology [5–8]. There are some recipients who are FCXM-positive despite being CDCXM-negative, most of whom also show vigorous antibody-mediated humoral rejection in spite of appropriate anti-rejection therapy. This type of antibody, which can be detected only by flow-cytometric analysis, and not CDCXM, could cause graft injuries independent of complement activation, through mechanisms such as

Table 5. HLA antibody specificities and their respective MESF value.

HLA antibody	MESF	Acceptable (yes/no)
B51	1421	Yes
	1369	Yes
	1759	Yes
B52	3563	No (AMR)
	920	Yes
A24	2152	No (AMR)
	3383	No (AMR)
DR10	4132	No (TMR, CAMR)
	4835	No (AMR, graft loss)
DR9	23 760	No (AMR, graft loss)
	29 030	No (AMR)
DR15	414 664	No (AMR)
	245 402	No (AMR)

HLA, human leukocyte antigen; MESF, molecules of equivalent soluble fluorochrome; AMR, antibody-mediated rejection; TMR, T-cell-mediated rejection; CAMR, chronic AMR.

antibody-dependent cell cytotoxicity (ADCC) with the help of effector cells like natural killer cells expressing FcR (Fc receptor). Thus, we discovered many immunologic aspects in cases of AMR by observing discrepancies in the results between CDCXM and FCXM.

Immunologic assay methods must be highly specific and highly sensitive. In addition, they should be standardized and highly reproducible so as to allow intra- and inter-experimental comparisons of results from different laboratories. The optimal method should be feasible and the results should become available within a matter of hours. Flow cytometry fulfills these prerequisites but the following issues have to be addressed: difficulties in quantitative evaluation of the target molecules; calibration of the instrument to compensate for variations in the environment, which might influence the sensitivity of the photomultipliers, etc. Development of methods that can be reproduced by any technician at any laboratory at any time is needed.

The use of beads with standard fluorescence, namely, MESF, was introduced by Schwarz, *et al.* [9] as a tool to compare quantitative MFI values over time and across platforms. In this study, we describe the results of quantitative MESF analysis in both subjects with DSA or NDSA.

As we expected, high DSA titers had a greater adverse influence on graft survival, while low DSA titers appeared to have no adverse effects. For example, patients with DSA with MESF values of over 3000 (8/11, 73%) showed a high incidence of AMR, while patients with DSA with MESF values of <3000 showed no evidence of rejection (10/11, 90%). The cutoff MESF value between the group that showed a high incidence of rejection and the group that showed no cases of rejection was thought to be approximately 3000 or so. In addition, the type of DSA

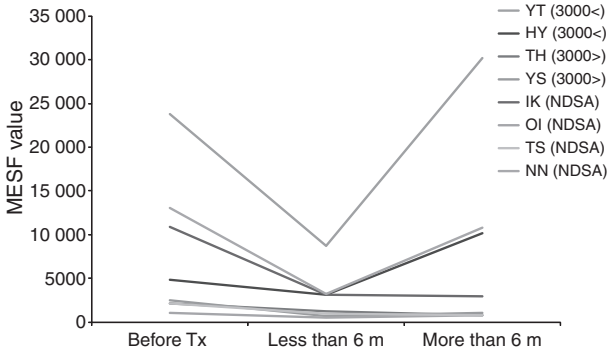

Figure 3 Change in molecules of equivalent soluble fluorochrome (MESF) value in representative patients before and after transplantation (Tx) (NDSA, nondonor-specific antibody).

was anti-HLA DR, a class II antibody, in 10 of the 22 patients of the DSA group (10/22, 45%), while the type of NDSA was a class II antibody, in two of the 12 patients of the NDSA group (2/12, 17%) (Table 3). Moreover, as shown in Table 5, MESF value of all class II (DR10, 9, 15) DSA were over 3000. Thus, in this study, class II DSA had a greater influence on graft rejection than class I DSA. Class II DSA is produced by activated B cells after indirect recognition mediated by CD4+ T cells [13]. Hourmant *et al.* [14] reported that among 68 recipients with DSA, all but one was class II.

Moreover, care should be taken in interpreting the biological significance of NDSA. Patients undergoing transplantation frequently have NDSA. In this study, the change in DSA and NDSA was observed before and after transplantation. Representative data are shown in Fig. 3. The DSA decreased and increased before and after transplantation respectively. Also, NDSA continuously and slowly decreased after transplantation, although the level initially increased in some patients. This pattern may be caused by the binding of antibodies with shared epitopes on donor-specific HLA antigens [15]. When DSA antibody production is small, DSA that has attached to the graft endothelium cannot be detected in the sera as DSA, and only NDSA is detected. In addition, natural HLA antibodies may also be found [16]. Natural antibodies, stimulated by microbial and colonial antigens, may include some with cross-reactivity to epitopes on some HLA molecules. However, the clinical significance of natural anti-HLA antibodies has yet to be determined.

Anti-HLA antibodies with high MESF values continue to exist in the recipient's sera at almost the same titers once AMR has occurred postoperatively. Within hours of re-exposure to the antigen after transplantation, memory B cells become activated plasmablasts, which convert to antibody-secreting plasma cells. Plasma cells are terminally differentiated and secrete antibody continuously, even in the absence of antigens. Some plasma cells migrate into the bone marrow and survive in this bone marrow niche as long-life plasma cells. These plasma cells in the bone marrow niche are resistant to antibody-mediated apoptosis *in vivo*; however, a recent study failed to show that ATG causes the apoptosis of marrow-derived CD138+ plasma cells *in vitro*. Some reports have discussed the effect of proteasome inhibitor against plasma cells derived from the bone marrow [17]. In general, conventional immunosuppressive agents have little or no effect on alloantibody production by plasma cells.

This study demonstrated that we could make more accurate predictions of graft survival by qualitative and quantitative evaluations of anti-HLA antibodies before kidney transplantation, and also that we could devise effective immunosuppressive protocols to treat patients with chronic rejection by effective assessment of the humoral activities in recipients. Further studies would be needed before quantitative MESF assay methods can be applied in clinical practice.

Authorship

HI: designed the research/study, performed research/study and wrote the paper. TH and NK: wrote the paper. YY: performed pathologic analysis. KT: contributed important reagents.

Acknowledgements

This study was supported by the International Research and Educational Institute for Integrated Medical Sciences, Tokyo Women's Medical University, which is supported by the Program for Promoting the Establishment of Strategic Research Centers, Special Coordination Funds for Promoting Science and Technology, Ministry of Education, Culture, Sports, Science and Technology (Japan).

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