Available online at www.annclinlabsci.org

Donor-Specific HLA Class I and CREG Antibodies in Complement-Dependent Cytotoxicity-Negative Renal Transplants

Yonggoo Kim,[1] Chul Woo Yang,[2] In-Sung Moon,[3] Myungshin Kim,[1] Jihyang Lim,[1] Yeon-Joon Park,[1] Kyungja Han,[1] and Eun-Jee Oh[1]
Departments of [1]Laboratory Medicine, [2]Internal Medicine, and [3]Transplantation Surgery, School of Medicine, The Catholic University of Korea, Seoul, Korea

Abstract. Development of a solid-phase, single antigen panel reactive antibody test (SA-PRA) permits the analysis of antibody specificities. This study determined the impact of donor-specific antibodies (DSA) against class I HLA private antigens (DS-HLA) or HLA-A and -B cross-reactive group (DS-CREG) in kidney transplantation. Pre- and post-transplant sera of 133 renal allograft patients who had negative pretransplant complement-dependent cytotoxicity were tested for HLA class I antibody specificities by SA-PRA. Clinical relevance of the flow cytometric crossmatch test (FCXM) for the detection of class I DS-HLA or DS-CREG was analyzed. The sensitivity of FCXM to detect SA-PRA-defined class I DSA was 50% (5/10) and the specificity was 98.4% (121/123). Of 133 renal allograft recipients, including 26 patients with biopsy-proven acute antibody-mediated rejection (AMR), pretransplant DS-HLA or DS-CREG were detected in 10 patients. Pretransplant DSA were associated with AMR ($p = 0.012$) and a low calculated glomerular filtration rate ($p = 0.036$). In the analysis of post-transplant sera, the presence of either type of HLA antibodies and the de novo development of DSA were correlated with AMR ($p < 0.001$). This study demonstrates that detection of DSA, including DS-HLA and DS-CREG, using the SA-PRA assay is useful to identify the renal allograft recipients with poor transplant outcome.

Keywords: acute antibody-mediated rejection, donor-specific HLA antibodies, renal transplantation

Introduction

The development of kidney graft rejection seems to be more frequent when HLA antibodies are donor-specific; any level of detectable donor-specific HLA antibodies is a risk to transplantation [1-4]. The anti-HLA antibodies detected in sera from sensitized patients are specific for epitopes shared by different HLA antigens (public epitopes), a cross-reactive antigen group (CREG), rather than private HLA antigens [5-8]. Although it has been reported that matching CREG is associated with an allograft survival rate similar to that obtained by matching HLA private antigens [9,10], the clinical impact of donor-specific CREG antibodies (DS-CREG) on renal graft rejection needs further investigation.

The detection of DSA has traditionally been performed with cell-based crossmatch tests, such as complement-dependent cytotoxicity (CDC) or flow cytometric crossmatching (FCXM). While the FCXM test has enhanced sensitivity compared with the CDC assay, HLA specificity should be determined before interpreting a positive result to rule out autoreactive non-HLA antibodies [11]. HLA antibodies are increasingly detected by solid-phase tests with purified HLA antigens attached either to microtiter plates or to beads. In particular, the panel reactive antibody test with single recombinant HLA antigens (single antigen PRA, SA-PRA) has been reported to be more sensitive

Address correspondence to Eun-Jee Oh, M.D, Ph.D, Department of Laboratory Medicine, School of Medicine, Catholic University, 505 Banpo-Dong, Seocho-Ku, 137-701, Seoul, Korea; tel 82 2 2258 1641; fax 82 2 2258 1719; e-mail ejoh@catholic.ac.kr.

0091-7370/10/0400-0330. $2.10.

and specific for HLA antibody detection than CDC and FCXM [12-14]. SA-PRA also enables assessment of DS-HLA antigens, DS-CREG antigens, and non-donor-specific HLA (NDS-HLA) immunization.

We investigated the DS-HLA and DS-CREG against HLA A-B- antigens in pre- and post-transplant sera of kidney-grafted recipients by SA-PRA ELISA assays. The goals of this study were to evaluate the influence of pre- and post-transplant DSA, including DS-HLA or DS-CREG, on transplant outcome and to analyze the clinical relevance of the FXCM test for detection of DSA.

Materials and Methods

Patients. The study population consisted of 133 consecutive patients who underwent ABO-compatible kidney transplantation at our center from January 2005 to June 2007. The 133 recipients had a mean age of 41 yr; 83 of the patients were males. Twenty-two patients (16.5%) received deceased donor transplants. Pretransplant CDC, FCXM, and PRA tests were performed and negative current T- and B-cell CDC crossmatch was required for all recipients. Immunosuppressive treatment consisted of a combination of prednisone, mycophenolate mofetil, and cyclosporine or tacrolimus. All patients received methylprednisolone (1 g/day, iv) on the day of transplantation, and then oral prednisolone was tapered down to 30 mg/day on the fourth day after transplantation.

Patients who had positive results in both pretransplant FCXM and PRA-ELISA tests received plasmapheresis and iv immunoglobulin treatment. HLA DNA typing information of donors and recipients was available to confirm that the detected HLA antibodies were specific for the mismatched HLA antigens of the graft. Delayed graft function was defined as the need for dialysis during the first week after transplantation. Postoperative protocol biopsies at 14 days posttransplant and episode biopsies were performed in all recipients. Acute antibody-mediated rejection (AMR) was diagnosed by histological examination and C4d staining of allograft biopsies using the Banff classification. The simplified Modification of Diet in Renal Disease (MDRD) formula was used to calculate the glomerular filtration rate (GFR) at 6 mo [15]. All recipients and donors gave written informed consent.

Flow-cytometric crossmatch. FCXM tests were performed as previously described [16]. Briefly, 2 x 10^5 donor lymphocytes and 50 µl of the patient serum were incubated for 30 min at room temperature. Fluorescein isothiocyanate-labeled anti-human IgG (DAKO, Kyoto, Japan) and phycoerythrin-labeled CD19 or CD3 (DAKO) were added for 30 min. After washing, the cells were analyzed using a Coulter EPICS XL flow cytometer (Beckman Coulter, Miami, FL). A positive FCXM test was defined as a shift of the mean channel fluorescence by >10 channels.

HLA antibody analysis. HLA antibodies were first detected by an ELISA screening test (LAT-M; One-Lambda, Canoga Park, CA). Additional identification of HLA specificity was performed using SA-PRA ELISA (LAT-single antigen; One-Lambda). SA-PRA consisted of 88 different HLA-A and -B alleles produced by recombinant technology. The SA-PRA ELISA test was performed as specified by the manufacturer. A positive reaction of the test serum was indicated by a reactivity score of 4, 6, or 8. According to the pretransplant HLA specificities, patients were classified into one of the following groups: (1) DS-HLA group with antibodies detected against private HLA class I specificity of mismatched donor HLA antigens, (2) DS-CREG group, who did not have reactivity against the private specificity of donor HLA antigens, but had antibodies directed against class I CREG of mismatched donor HLA antigens, (3) NDS-HLA group, who had anti-HLA antibodies other than against CREG or mismatched donor HLA antigens, and (4) non-HLA immunization groups (No-HLA), who did not have anti-HLA antibodies. CREG were defined according to Rodey et al [5]. Patients who had DS-HLA with DS-CREG or NDS-HLA were included in the DS-HLA group. One-to-three sera for each patient were tested in the posttransplant period (1 wk to 6 mo) and the peak PRA result was chosen.

Statistical analyses were performed with SPSS 10.1 and differences between groups were evaluated by Fischer's exact test and the Mann-Whitney U-test. A p value of ≤0.05 was deemed statistically significant.

Results

Pretransplant FXCM test and specificity of class I HLA antibodies. Of 133 recipients, 26 patients (19.5%) had an AMR and 7 had graft failure. The FCXM test was positive in 7 patients (5.3%), including 1 patient with B-cell-positivity alone and 6 patients with both T- and B-cell-positivity (Table 1). According to pretransplant SA-PRA tests, 15 (11.3%) of 133 recipients had HLA class I antibodies (4 [3.0%] DS-HLA, 6 [4.5%] DS-CREG, and 5 [3.8%] NDS-HLA) and 118 (88.7%) had no HLA antibodies. Among 126 patients with T- and B-cell FCXM-negativity, 5 patients were found to have DS-HLA or DS-CREG. In 2 patients with T- and B-cell FCXM-positivity, no HLA antibodies were detected by the SA-PRA test. The sensitivity of the FCXM test to detect DS-HLA or DS-CREG was 50.0% (5/10) and specificity was 98.4% (121/123). In FCXM-negative patients, presence of DS-HLA or DS-CREG was associated with a posttransplant AMR ($p = 0.042$). Of the 4 patients who were FCXM-negative and DS-CREG positive, 3 patients (75%) developed posttransplant AMR.

Table 1. Results of the pretransplant FCXM test and class I HLA antibody specificity by single antigen PRA in the 133 renal transplant recipients.

FCXM	Class I HLA antibodies	No. (%)	No. (%) of Acute Rejection
T (-) B (-), n = 126	DS-HLA	1 (0.8)	0 (0.0)
	DS-CREG	4 (3.2)	3 (75.0)
	NDS-HLA	5 (3.9)	1 (20.0)
	No-HLA	116 (92.1)	19 (16.4)
T (-) B (+), n = 1	DS-HLA	1* (100)	0 (0.0)
T (+) B (+), n = 6	DS-HLA	2* (33.3)	1 (50.0)
	DS-CREG	2* (33.3)	1 (50.0)
	No-HLA	2* (33.3)	1 (50.0)

*Patients who received pretransplant plasmapheresis and intravenous immunoglobulin.

Table 2. Clinical characteristics of the 133 renal transplant recipients according to pretransplant class I HLA antibodies.

Variable	DS-HLA and DS-CREG (-) (n = 123)	DS-HLA or DS-CREG (+) (n = 10)	p value
Recipient age (yr, mean ± SD)	41 ± 11	42 ± 8	0.729
Recipient male gender	78 (63.4%)	5 (50.0%)	0.401
Donor age (yr, mean ± SD)	39 ± 11	36 ± 14	0.571
Donor male gender	60 (48.8%)	4 (40.0%)	0.594
Deceased donor	19 (15.4%)	3 (30.0%)	0.215
HLA mismatches (≥4)	49 (39.8%)	4 (40.0%)	0.991
Delayed graft function (+)	11 (8.9%)	3 (30.0%)	0.072
FCXM-positive	2 (1.6%)	5 (50.0%)	<0.001
Acute rejection	21 (17.1%)	5 (50.0%)	0.012
MDRD 6 mo posttransplant (mean ± SD)	66.2 ± 19.3	54.9 ± 10.2	0.036
Graft failure	5 (4.1%)	2 (20%)	0.087

Table 3. Posttransplant class I antibody specificities in 12 renal transplant recipients.

Patients	Gender		Antibody specificity			AMR	Graft failure
			DS		NDS		
			DS-HLA	DS-CREG*			
1	M	Persistent	A24			+	+
2	F			1C		+	+
3	F			7C		+	
4	F			12C		+	+
5	F			1C		+	+
6	M	de novo	A2			+	+
7	F		A2			+	
8	F		A11			+	
9	M			5C		+	
10	M			1C		+	
11	F			5C, 7C		+	
12	F				A33	+	

*CREG were defined according to reference [5].

Table 4. Results of the pre- and post-transplant class I HLA antibody specificities and acute antibody-mediated rejection (AMR) in the 133 renal transplant recipients.

Pre-transplant DSA	Post-transplant DSA	No. (%) of patients	No. (%) of AMR(+)
N	N	117 (87.9)	15 (12.8)
N	P	6 (4.5)	6 (100)
P	N	5 (3.8)	0 (0)
P	P	5 (3.8)	5 (100)

Detection of pretransplant DSA. When we divided the patients into both pretransplant DS-HLA- and DS-CREG-negative groups (pre-KT DSA-; n = 123) and pretransplant DS-HLA- or DS-CREG-positive group (pre-KT DSA+; n = 10), there were no significant differences in pretransplant baseline characteristics, including age, gender, deceased donor, HLA mismatch, and delayed graft function between the two groups (Table 2). The pre-KT DSA+ patients experienced higher frequency of AMR than the pre-KT DSA- patients (50% [5/10] vs 17.1% [21/123], respectively, p = 0.012). The calculated GFR (MDRD) at 6 mo was lower in the pre-KT DSA+ group than in the pre-KT DSA- group (54.9 ± 10.2 vs 66.2 ± 19.3 ml/min, p = 0.036). Among the 5 patients who had pretransplant NDS-HLA antibodies, only one patient experienced an AMR (Table 1).

Detection of post-transplant DSA. With respect to post-transplantation antibodies, HLA class I antibodies were detected in 12 recipients (4 DS-HLA, 7 DS-CREG, 1 NDS-HLA). Of the 11 patients with post-transplant DSA, 5 patients had persistent antibodies and 6 patients developed de novo donor-specific HLA antibodies (3 DS-HLA and 3 DS-CREG) (Table 3). All of the 11 patients with post-KT DSA had an AMR and 42.3% (11/26) of the patients who developed AMR presented with post-transplant DSA. The patients with de novo anti-HLA antibodies had a higher incidence of AMR (100% vs 11.6%), and more allograft failures (50% vs 1.7%) compared to patients without post-transplant anti-HLA antibodies. The presence of either type of HLA antibodies in the post-transplant period was correlated with an AMR (p <0.001). In analyzing the development of AMR with de novo DSA after transplantation, all 5 patients with pre-KT DSA(-)/post-KT DSA(+) experienced AMR and the 5 patients with pre-KT DSA(+)/post-KT DSA(-) did not develop AMR (Table 4). Of the 133 patients, only 1 patient developed post-KT de novo NDS-HLA antibodies and experienced an AMR.

Discussion

The high polymorphism of the major histocompatibility complex is reflected in the multiple polymorphic determinants, called CREG [17]. Alloimmunized patients make HLA antibodies to the HLA antigens that share many more structural similarities than differences related to the highly polymorphic private HLA antigens [18]. Therefore, the identification of HLA antibodies based on the CREG can improve the clinical outcome of kidney allografts. In previous reports, CREG matching and CREG classification was directed against the class I HLA-A, -B rather than against class II HLA [5-10]. As we intended to evaluate the impact of DSA including both DS-HLA and DS-CREG, we included only class I antibodies in this study. Because AMR remains a major cause of morbidity for renal transplants after transplantation, we determined whether pre- or post-DSA against class I HLA antigens or CREG could predict early AMR and graft dysfunction.

Presence of DSA causes a positive crossmatch test and graft allocation depends on the current lymphocytotoxicity test. The FCXM test has been shown to be a more sensitive and specific method than the CDC test and it also distinguishes IgG from IgM antibodies. In this study, among the 133 patients who received negative CDC crossmatch kidneys, pretransplant FCXM tests were positive in 7 patients (5.3%). Comparison of FCXM testing results and DSA by SA-PRA indicates that FCXM testing gave false positive results in 2 patients and false negative results in 5 patients. As solid-phase

based technologies may be more sensitive and specific than cell-based tests in detecting antibodies to HLA antigens, a final crossmatch test is recommended to approximate the sensitivity of the solid-phase assay [19]. Therefore we chose SA-PRA as a gold standard in comparison with FCXM.

Solid-phase PRA tests can be grouped according to the number of HLA antigens coated on the solid phase as multiple antigens (HLA antigens pooled from many individuals), several antigens (purified HLA antigens from one individual), and single antigens [20,21]. The resulting antibodies from PRA tests with multiple antigens or several antigens may be highly cross-reactive, and determination of antibody specificity is difficult in highly sensitized recipients.

Allograft rejection is caused by several elements of the immune system, including antibody, complement, T cells and other cell types [22]. T cell mediated injury has been considered to be the main cause of kidney transplantation rejection and T cell directed immunosuppression has decreased the incidence of acute T cell mediated rejection. However, AMR is increasingly recognized with differential effects and several studies have shown an association between DSA and chronic rejection. In this study, we used SA-PRA to accurately characterize the HLA class I antibodies, and the presence of pretransplant DSA was significantly associated with the development of an AMR and low MDRD at 6 mo ($p = 0.012$ and $p = 0.036$, respectively). Although previous studies [1,2] showed a strong association between DSA and poor graft outcome, it is important to emphasize that the SA-PRA allowed us to identify most of the antibodies specific for CREG.

Routine monitoring for DSA is still not performed at many centers. However, posttransplant immune evaluation of those recipients would allow possible intervention or other therapeutic strategies to prevent graft failure [11]. In this study, 6 patients developed de novo anti-HLA antibodies after transplantation and 3 of them developed DS-CREG. This finding supports previous observations [7,8] that most anti-HLA class I antibodies detected in serum samples from sensitized patients are specific for CREG. In this study, all the patients with de novo anti-HLA antibodies experienced an AMR and half of them had allograft failure. These findings are in agreement with previous reports [23-25] in which the development of de novo anti-HLA antibodies was associated with acute rejection, emphasizing the need of post-transplant monitoring of HLA specific antibodies to improve graft outcome. In this study, the one patient with de novo NDS-HLA after transplantation had an AMR and the clinical significance of NDS-HLA antibodies is not clear. Further studies with a larger population of renal transplant patients are therefore required.

Two generations of solid phase assays have been introduced into the clinical laboratory, ELISA and luminex. The luminex assay has become the most popular approach and has been shown to be more sensitive than ELISA [26]. Because this study was performed using the ELISA method, we need to establish comparisons with luminex technology.

In summary, our study demonstrates the clinical significance of identifying pre- and post-transplant DSA including DS-HLA or DS-CREG in CDC-negative kidney transplants. Post-transplantation monitoring for detection of DSA can be effectively performed using the SA-PRA.

Acknowledgement

This study was supported by grant A092258 from the Korean Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea.

References

1. Worthington JE, Thomas AA, Dyer PA, Martin S. Detection of HLA-specific antibodies by PRA-STAT and their association with transplant outcome. Transplantation 1998;65:121-125.
2. Schönemann C, Groth J, Leverenz S, May G. HLA class I and class II antibodies: monitoring before and after kidney transplantation and their clinical relevance. Transplantation 1998;65:1519-1523.
3. Süsal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. Transplantation 2002;73:1269-1273.
4. Magee CC. Transplantation across previously incompatible immunological barriers. Transpl Int 2006;19:87-97.
5. Rodey GE, Neylan JF, Whelchel JD, Revels KW, Bray RA. Epitope specificity of HLA class I alloantibodies. I. Frequency analysis of antibodies to private versus public

specificities in potential transplant recipients. Hum Immunol 1994;39:272-280.
6. Piazza A, Poggi E, Ozzella G Borrelli L, Monaco PI, Scornajenghi A, Tisone G, Adorno D. Public epitope specificity of HLA class I antibodies induced by a failed kidney transplant: alloantibody characterization by flowcytometric techniques. Transplantation 2006;81: 1298-1305.
7. Barocci S, Valente U, Nocera A. Detection and analysis of HLA class I and class II specific alloantibodies in the sera of dialysis recipients waiting for a renal retransplantation. Clin Transplant 2007;21:47-56.
8. Cha CH, Oh HB, Kim MH, Chae JM, Jung SY. Development of a web-based program for the identification of human leukocyte antigen antibody specificities Korean J Lab Med 2007;27:458-463.
9. Egfjord M, Jakobsen BK, Ladefoged J. No impact of cross-reactive group human leucocyte antigen class I matching on long-term kidney graft survival. Scand J Immunol 2003;57:362-365.
10. Laux G, Opelz G. Immunological relevance of CREG matching in cadaver kidney transplantation. Transplantation 2004;78:442-446.
11. Zachary AA, Leffell MS. Detecting and monitoring human leukocyte antigen-specific antibodies. Hum Immunol 2008;69:591-604.
12. Magee B, Martin J, Middleton D. The repercussions of implementing flowcytometry as a single HLA antibody screening technique in prospective renal transplant recipients. Transpl Int 2006;19:105-109.
13. El-Awar N, Lee J, Terasaki PI. HLA antibody identification with single antigen beads compared to conventional methods. Hum Immunol 2005;66:989-997.
14. Pei R, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation 2003;75:43-49.
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-470.
16. Yang CW, Oh EJ, Lee SB, Moon IS, Kim DG, Choi BS, Park SC, Choi YJ, Park YJ, Han K. Detection of donor-specific anti-HLA class I and II antibodies using antibody monitoring system. Transplant Proc 2006;38:2803-2806.
17. Parham P, Adams EJ, Arnett KL. The origins of HLA-A,B,C polymorphism. Immunol Rev 1995;143:141-180.
18. Laundy GJ, Bradley BA. The predictive value of epitope analysis in highly sensitized patients awaiting renal transplantation. Transplantation 1995;59:1207-1213.
19. Gebel HM, Bray RA, Nickerson P. Pretransplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. Am J Transplant 2003;3:1488-1500.
20. Schaub S, Scornik JC. High-tech detection of HLA antibodies and complement: prospects and limitations. Am J Transplant 2008;8:5-6.
21. El-Awar N, Lee JH, Tarsitani C, Terasaki PI. HLA class I epitopes: recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. Hum Immunol 2007;68:170-180.
22. Cornell LD, Smith RN, Colvin RB. Kidney transplantation: mechanisms of rejection and acceptance. Annu Rev Pathol 2008;3:189-220.
23. Zhang Q, Liang LW, Gjertson DW, Lassman C, Wilkinson AH, Kendrick E, Pham PT, Danovitch GM, Gritsch HA, Reed EF. Development of posttransplant antidonor HLA antibodies is associated with acute humoral rejection and early graft dysfunction. Transplantation 2005;79:591-598.
24. Cecka JM. The role of HLA in renal transplantation. Hum Immunol 1997;56:6-16.
25. Li X, Ishida H, Yamaguchi Y, Tanabe K. Poor graft outcome in recipients with de novo donor-specific anti-HLA antibodies after living related kidney transplantation. Transpl Int 2008;21:1145-1152.
26. Tait BD. Solid phase assays for HLA antibody detection in clinical transplantation. Curr Opin Immunol 2009; 21:573-577.