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Anti-HLA Donor-Specific IgG Subclasses and C1q-binding Evolution in Posttransplant Monitoring

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Background. The identification of low-level antibodies by single-antigen bead methodology has brought advancements to risk evaluation of kidney transplant recipients. However, the use of mean fluorescence intensity (MFI) to quantify antibodies and to guide therapy is not enough. Notably, immunoglobulin G (IgG) subclass switching is hypothesized to follow a programmed sequence after an emergency signal from the germinal center. In transplantation this process is not clear yet. In the present study, we sequentially evaluate anti-HLA donor specific antibody (DSA) subclasses, their profile changes, and C1q-binding ability and the influence of those characteristics on antibody mediated rejection (AMR) occurrence and allograft function. **Methods.** A total of 30 DSA-positive patients were tested for IgG subclass content and C1q-binding in sequential serum samples. **Results.** Twenty-one patients were DSA-positive before transplant; patients sensitized only by transfusion or pregnancies had IgG1 and/or IgG3, and patients sensitized by both transfusion and pregnancies or previous transplant showed a broader range of IgG subclasses. C1q binding was detected in high MFI made up of IgG1 or multiple IgG subclasses. Only 4 patients were positive for C1q posttransplantation and 3 of these showed an increase in MFI, changes in subclasses patterns, AMR, and allograft dysfunction. **Conclusions.** Posttransplant evaluation of DSA subclasses and the ability to bind C1q may be informative for both AMR occurrence and allograft dysfunction. Monitoring these events may help to better define risk and interventional time points.

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Technological advancements in antibody identification have revolutionized how we assess risk in solid organ transplantation. From the identification of low-level antibodies to the characterization of highly sensitized retransplant patients,[1] these advancements, particularly solid phase single-antigen beads (SABs) assays, have made it exponentially easier to differentiate and categorize patients.[2,3] However, as beneficial as this is to organ allocation and desensitization protocols,[4] it provides minimal improvement in rejection diagnosis and treatment,[5,6] particularly when attempting quantification through mean fluorescence intensity (MFI).[5]

Although there is an association of elevated MFI values with worse outcomes, there is very little evidence supporting a direct correlation of MFI and clinical impact. For example,

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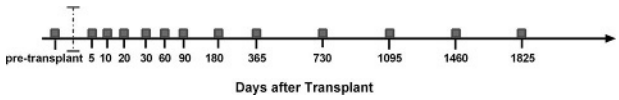

FIGURE 1. Sera samples collected during prospective posttransplant monitoring.

Lefaucheur et al[7] showed in a pretransplant setting that patients with donor-specific antibody (DSA) higher than 6000 MFI presented a 100-fold increased risk for antibody-mediated rejection (AMR) (relative risk, 113.0; 95% confidence interval [CI], 30.8-414), however, the CI showed by the data suggests that some of the patients in this group presented lower risk for AMR than patients with MFI between 465 and 1500 (relative risk, 24.8; 95% CI, 4.6-134.8. When evaluating large cohorts, it is possible to find greater risk associated to higher MFI values, but the question remains, what differs in patients with high MFI values that develop AMR versus those that do not?[5,8]

In the posttransplant setting, the appearance of anti-HLA DSAs, as determined by MFI and the subsequent rise and/or fall of the MFI value, although implying risk, does very little to define the function and activity of that antibody.[8,9] When considering the functionality of DSA, it was recently demonstrated by Loupy et al[10] that allograft survival in the presence of C1q-binding DSA was significantly lower than that in patients with non–C1q-binding DSA and no DSA. However, in their cross-sectional analysis of 1016 patients tested at 1 year or at the time of rejection with a 5-year follow-up, they found only 77 patients with C1q-positive DSA. Although providing a highly significant cohort of patients at higher risk for allograft loss, suggesting the functionality of DSA at a specific time point, these data do little to elucidate the true evolution of the immune response. One could argue that instead of being a marker for rejection and possibly an opportunity for intervention, it is nothing more than evidence of a predetermined fate.

It has recently been shown that the presence of complement-fixing IgG (IgG1 and/or IgG3) is abundant in kidney transplant patient serum but is not a determinant of the detection of C1q-binding.[11] Moreover, subclass switching is hypothesized to follow a programmed sequence after an emergency signal from the germinal center leading to the production of IgM followed by IgG3, then IgG1, then IgG2, and finally IgG4.[12] This process is influenced by the initial immune response, the microenvironment of cytokines, and the signaling produced.[13,14] There are many reports suggesting that IgG3 and IgG1 appear relatively early in the immune response and are often the only subclasses detected, which could mean early antigen clearance.[12] Arnold et al[15] observed that AMR features were more common in patients

TABLE 1.

Patient demographics

Characteristics		All patients	DSA+C1q–	DSA+C1q+	*P*
N total	n (%)	30 (100)	23 (76.7)	7 (23.3)	
Female	n (%)	17 (56.7)	12 (52.2)	5 (71.4)	0.38
Age, y	Mean ± SD	42.17 ± 13.70	41.09 ± 13.82	45.71 ± 13.68	0.44
Transplant type					
Live unrelated	n (%)	12 (40.0)	8 (34.8)	4 (57.1)	0.30
Live related	n (%)	18 (60.0)	15 (65.2)	3 (42.9)	
Previous sensitization					
Transfusions	Median (min-max)	2.0 ± 2.9	2.1 ± 3.2	1.8 ± 2.2	0.85
Pregnancies, n = 17	Median (min-max)	3 (0-19)	2.5 (0-19)	3.0 (2-9)	0.38
Regrafts	n (%)	3 (10.0)	3 (13.0)	0	0.32
HLA compatibility					
No. mismatches	mean ± SD	4.23 ± 1.48	4.2 ± 1.6	4.4 ± 1.1	0.70
PRA	mean ± SD	56.6 ± 39.8	54.9 ± 38.4	62.1 ± 46.8	0.68
Clinical events					
Induction therapy	n (%)	17 (56.7)	15 (65.2)	2 (28.6)	0.09
Dialysis time, d	Median (min-max)	730 (0-4380)	700 (0-3650)	1278 (153-4380)	0.22
Diabetes	n (%)	1 (3.3)	1 (4.3)	0	0.58
Hypertension	n (%)	20 (66.7)	15 (65.2)	5 (71.4)	0.76
Follow-up time, d	Median (min-max)	1801.5 (289-2176)	1833 (951-2176)	1770 (289-1914)	0.33
Infection	n (%)	20 (66.7)	15 (65.2)	5 (71.4)	0.76
Graft loss	n (%)	2 (6.7)	0	2 (28.6)	0.009
Patient death	n (%)	3 (10.0)	2 (8.7)	1 (14.3)	0.67
Histological factors					
AMR	n (%)	4 (13.3)	0	4 (57.1)	0.0001
TCMR	n (%)	2 (6.7)	2 (8.7)	0	0.63
CsA nephropathy	n (%)	1 (3.3)	1 (4.3)	0	0.58
BKV nephropathy	n (%)	4 (13.3)	4 (17.4)	0	0.24

χ^2 tests were used for comparison of categorical variables and 1-way ANOVA was used for the comparison of parametric continuous variables. Comparison between groups of nonparametric variables was performed by the Kruskal-Wallis method. Nonparametric variables are presented as the median (range).

BKV, BK virus; CsA, cyclosporine; min, minimum; max, maximum.

with an expansion to non–complement-fixing DSA. This suggests that the expansion of complement-fixing to non–complement-fixing DSA shows an evolution of the immune response. Little has been described in transplantation about this process, because most studies evaluate pretransplant sera alone or pretransplant sera with only 1 posttransplant time point.[16-21]

In the present study, we sequentially evaluated anti-HLA DSA subclasses, their profile changes, and C1q-binding ability while observing the influence of those characteristics on AMR and allograft function in live donor kidney transplant recipients.

METHODOLOGY

Patients and Sera Selection

From January of 2007, we prospectively monitored kidney transplant recipients for the presence of donor-specific anti-HLA antibodies (DSA) by SAB. Between 2007 and 2010, 158 patients received kidneys from live donors at the Hospital Universitário Evangélico de Curitiba. From those, 63 patients were excluded due to lack of complete follow-up or available serum for new tests, and 65 did not present DSA during the 5 years of posttransplant monitoring. Thirty patients were included in the study, 21 with preformed DSA and 9 with de novo DSA. All transplants required a negative complement-dependent cytotoxicity crossmatch for IgG T cell and B cell, and ABO blood group compatibility. Sera collection is shown in Figure 1. The study was approved by the Ethics Committee for Research from the Pontifícia Universidade Católica do Paraná.

Clinical Data

Clinical data on donors and recipients were obtained from the original medical records. Immunosuppression included prednisone, cyclosporine, and mycophenolate mofetil. Acute clinical rejection was characterized by deterioration of allograft function, proteinuria, and histopathological evidence. Allograft function is shown as estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) formula in mL/min per 1.73 m^2. Proteinuria is presented in the following categories: P+, 150 to 500 mg; P++, 500 to 1000 mg; P+++, 1000 to 3500 mg; P++++, >3500 mg. Biopsies were reanalyzed by 2 different pathologists without any clinical information of the recipient and were classified according to the most recent Banff classification criteria. C4d was performed for all biopsies.

Detection of IgG and DSA Characterization

Pretransplant and posttransplant sera were tested for class I and class II anti-HLA antibodies (SAB-IgG_{total}) with commercially available, Luminex-based Single Antigen Bead assay kits (LABScreen Single Antigen LS1A04 and LS2A01; One Lambda, Inc.; Canoga Park, CA) per the manufacturer's protocol and analyzed with HLA FUSION software (One Lambda, Inc.). A positive result was defined as a baseline normalized mean fluorescence intensity (MFI) greater than 500. Donor specificity for anti-HLA antibodies was determined by the comparison of the HLA antibody specificities with the HLA typing of the donor for HLA-A, -B, -DRB1, -DRB3, -4 and -5, -DQB1, and -DQA1 loci. HLA typing for both patient and donor was performed by LABType SSO (One Lambda, Inc.).

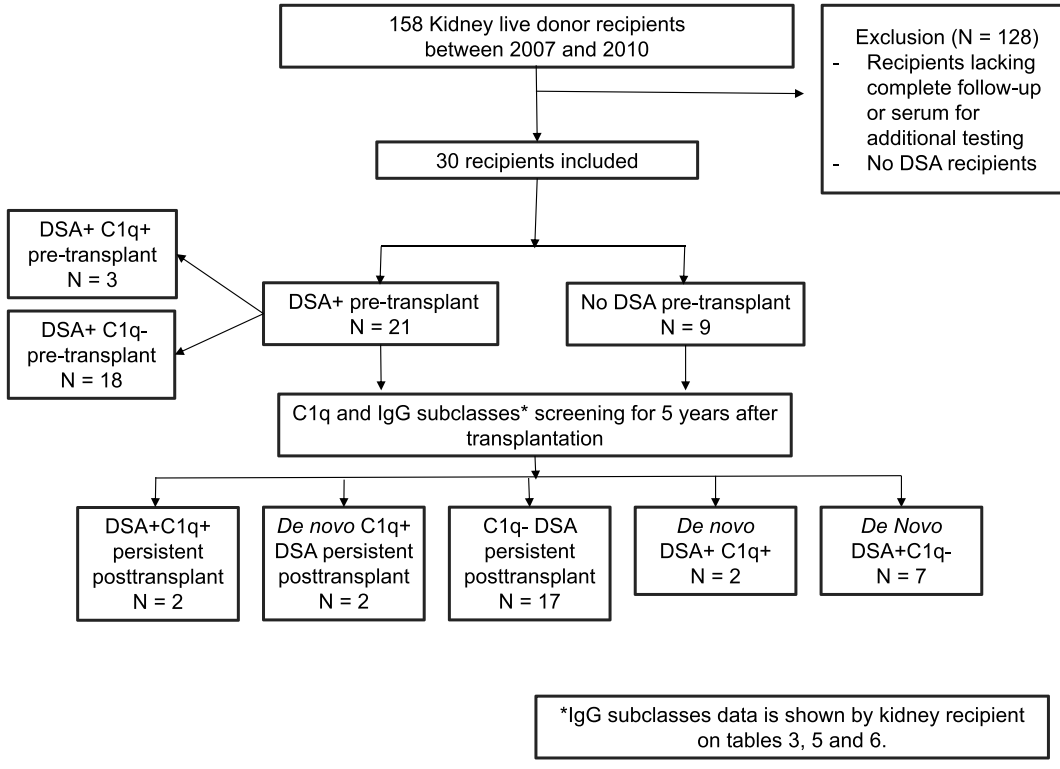

FIGURE 2. Study population according to anti-HLA DSA and C1q-binding status. C1q+-C1q-binding positive; C1q–-C1q-binding negative.

Detection of IgG Subclasses

DSA-positive samples were tested with a modified SAB assay to determine the IgG subclass of the antibody. The generic secondary antibody IgG_{total} (One Lambda, Inc.) was replaced by monoclonal secondary antibodies specific for IgG_{1-4} subclasses conjugated with phycoerythrin (PE) (IgG_1 clone HP6001, IgG_2 clone 31-7-4, IgG_3 clone HP6050, IgG_4 clone HP6025; Southern Biotech, Birmingham, AL). A positive result was defined as an MFI above the cutoff ratio that was generated for each individual bead of each subclass using 3 negative control sera (NC1-3) obtained from healthy, nonsensitized, anti-HLA antibody negative males, and 1 commercially available negative control serum (NC4). The cutoff ratio was calculated as follows: cutoff MFI = 2 × ((mean NC1-4) + 3 × (Standard deviations NC1-4)); cutoff ratio = MFI/cutoff MFI. A ratio above 5 was considered positive.

Detection of Complement-fixing Antibodies

DSA-positive samples were tested for C1q-binding anti-HLA antibodies (SAB-C1q) using commercially available kits (C1qScreen; One Lambda). The serum samples were heat-treated (56°C for 30 minutes) to denature endogenous complement components and the test was performed per the manufacturer's protocol. The analyses of C1q results were performed by HLA FUSION software (One Lambda, Inc.) following the interpretation method published by Tyan et al.[22]

Statistics

Comparison of patient and donor characteristics between groups according to sensitization status was performed with the χ^2 test for discrete variables and with analysis of variance (ANOVA) or the Kruskal-Wallis test, as applicable, for quantitative variables. Survival was analyzed from the time of transplantation to a maximum of 5 years using kidney allograft loss as the event of interest. Survival rates were compared according to the presence of anti-HLA DSA C1q-binding status using the log-rank test. Allograft function was evaluated by comparing the means of groups with DSA C1q-binding and DSA without C1q-binding by ANOVA 1-way on the following posttransplant days: 30, 180, 365, 730, 1095, 1460, and 1825. We further applied repeated-measures ANOVA to evaluate the variance in eGFR MDRD over time after transplant according to the presence of DSA C1q-binding. All statistical analyses were performed using MedCalc for Windows, version 13.0 (MedCalc Software, Ostend, Belgium). Statistical significance was set at P less than 0.05.

RESULTS

Study Population Demographics

Patient characteristics according to C1q DSA status are shown in Table 1. Patients with C1q DSA-positive at any time during the study were included in the group DSA+C1q+. We tested a mean of twelve serum samples per patient from pretransplant up to 5 years after transplantation for the presence of DSA. In the posttransplant evaluation, anti-HLA class II DSA was more frequent than class I alone and classes I and II together. IgG subclasses were primarily found in combinations, IgG1 being the most frequent. C1q-binding was detected in 3 pretransplant patients, 2 of whom

TABLE 2.

Mean fluorescence intensity in SAB IgG total and SAB C1q by groups of IgG subclasses

Anti- HLA antibody IgG subclass composition	No subclass	IgG1	IgG2	IgG3	IgG4	IgG1 + IgG2	IgG1 + IgG3	IgG1 + IgG4	IgG3 + IgG4	IgG2 + IgG4	IgG1 + IgG2 + IgG3	IgG1 + IgG2 + IgG4	IgG1 + IgG3 + IgG4	IgG1 + IgG2 + IgG3 + IgG4
No. anti-HLA class I IgGt-positive beads[a]	630	441	17	78	39	96	121	37	22	1	5	1	25	85
Mean MFI IgGt class I	1780	4931	4512	7610	1664	6411	8882	1938	2981	1902	8530	2518	9292	10310
Mean MFI C1q class I	57	790	49	1273	64	1538	6433	89	234	43	3472	70	3125	5751
No. anti-HLA class II IgGt-positive beads[a]	899	511	1	39	54	123	65	123	2	1	68	110	3	88
Mean MFI IgGt class II	2830	6414	2916	2136	2619	10112	6883	9843	2040	9364	9140	10547	11033	6604
Mean MFI C1q class II	31	1040	36	58	40	5748	4881	682	22	22	10422	4516	1585	9308

[a] All beads with mean fluorescence intensity above 500 were considered.

IgGt, Immunoglobulin G total.

TABLE 3.

Pretransplant antibody profile

	Pretransplant antibody profile					
Patient ID	**Sensitization**	**PRA (%)**	**HLA class**	**Anti-HLA Antibodies (IgGt MFI-IgG subclass)**	**Anti-HLA Antibodies IgGt+ with no subclass (MFI)**	**C1q binding (MFI)**
1	Transfusion	76%	II	**DQ7(3)* (2934-IgG1)**		Negative
2	Not Known	44%	I	**B12* (2121-IgG1)** B8 (2297 -IgG1) B37 (1851-IgG1 + IgG2) B41 (2666-IgG1) B42 (2609-IgG1) B82 (4529-IgG1)		**B12* (3083)** B37 (4001) B82 (6173)
3	Transfusion +Pregnancies	84%	II	**DR53* (4717-IgG3)**	**DR9* (4547)** DR18 (738) DR7 (4471) DR52 (1544) DQA1*03:01 (621) DQB1*03:02/DQA1*01:01 (2613)	Negative
4	Transfusion +Pregnancies	97%	I and II	**A9* (2238-IgG1)** B27 (719-IgG1) B39 (743-IgG1) B45(728-IgG1) B21 (677) B22 (1140) B67 (691-IgG1 + IgG4) **DR3* (6255-IgG1 + IgG2)** DR4 (1259-IgG4) DR7 (3372-IgG1 + IgG4) DR8 (3188-IgG1) DR9 (2905-IgG1) DR5 (5626-IgG1 + IgG2 + IgG4) DR6 (8787-IgG1 + IgG2) DR52 (3826-IgG1 + IgG4) DQB1*03:01/DQA1*05:03/DQA1*05:05 (927-IgG4)	**A10* (699) B8* (1268)** B14 (1165) B15 (677) B70 (661) B42 (964)B81 (676) B82 (801)	Negative
5	Previous Transplantation	98%	II	**DR4* (7611-IgG1 + IgG2 + IgG4) DR53* (12593-IgG1 + IgG2 + IgG4)** DR1 (5963-IgG1 + IgG2 + IgG4) DR7 (13794-IgG1) DR9 (11016-IgG1 + IgG4) DR10 (8489-IgG1 + IgG4) DR14 (6518-IgG1) DR15(2) (9958-IgG1 + IgG2 + IgG4) DR16(2) (9434-IgG1 + IgG4) DR51 (13501-IgG1 + IgG2 + IgG4) DR12 (3498-IgG1) DQB1*05:01/DQA1*01:01 (6696-IgG1 + IgG2 + IgG4) DQB1*05:02/DQA1*01:02 (6056) (IgG1 + IgG2 + IgG4)		DR1 (5901) DR7 (1171) DR9 (1436) DR10 (1225) DR15(2) (726) DR16(2) (772) DR51 (1088) DQB1*05:01/DQA1*01:01 (2209)

Continued next page

TABLE 3. (Continued)

				Pretransplant antibody profile		
Patient ID	**Sensitization**	**PRA (%)**	**HLA class**	**Anti-HLA Antibodies (IgGt MFI-IgG subclass)**	**Anti-HLA Antibodies IgGt+ with no subclass (MFI)**	**C1q binding (MFI)**
6	Transfusion +Pregnancies	99%	I	**A1* (3502-IgG1)** A2 (6686-IgG1 IgG2 + IgG3) A3 (5745-IgG1) A11 (4895-IgG1) A9(6821-IgG1 + IgG2 + IgG3) A10 (5367-IgG1 + IgG2) A*3201 (5550-IgG1 + IgG2) A19 (2967-IgG1) A36 (2967- IgG1) A28 (12043-IgG1 + IgG2 + IgG3) A80 (1640-IgG1) B13 (3633-IgG1 + IgG2) B15 (4174-IgG1 + IgG2) B27 (4158-IgG1 + IgG2) B37 (2879-IgG1) B16 (3972-IgG1 + IgG2) B39 (1866-IgG1) B12 (3455-IgG1 + IgG2) B47 (2999-IgG1) B21 (6413-IgG1 + IgG2) B5 (3771-IgG1 + IgG2) B53 (4898-IgG1 + IgG2) B17 (7543-IgG1 + IgG2) B59 (3475-IgG1 + IgG2)		**A1* (5011)** A2(11698) A3 (7811) A11 (3735) A9 (8866) A10 (928) A19(1193) A36 (4659) A28 (12043) A80 (868) B15 (1203) B37 (777) B16 (1821) B5 (1408) B53 (1137) B17 (6075) B59 (1019)
7	Transfusion	40%	I	**B7* (3998-IgG1)** B27 (2650-IgG1) B81 (3915-IgG1)	B13(1008) B40 (939) B42 (2059) B48 (1122) B22 (2530) B67 (738)	Negative
8	Transfusion +Pregnancies	80%	I	**A28* (5207-IgG1 + IgG2 + IgG3)** A2 (10752-IgG1 + IgG2 + IgG3 + IgG4) B15 (IgG1 + IgG2) B12 (4339-IgG1 + IgG2) B45 (10609-IgG1 + IgG2 + IgG4) B17 (2533-IgG1) B82 (7116-IgG1 + IgG2)	B13 (964) B21(1996)	A2 (327)
9	Transfusion	86%	I	B7 (3951-IgG1 + IgG3) B27 (1743-IgG1 + IgG3) B40 (935-IgG3) B42 (1760-IgG3) B48 (1208-IgG3) B81 (3746-IgG1 + IgG2)	**B13* (665)** A10 (667) B15 (818) B12 (940) B22 (637) B67 (1486) B82 (916)	Negative
10	Transfusion +Pregnancies	72%	I	**A1* (3689-IgG1 + IgG3)** A3 (1437-IgG1 + IgG3) A11(1540-IgG1 + IgG3) A9 (1195-IgG1 + IgG3) A36 (2431-IgG12 + IgG3) A80 (1587-IgG1 + IgG3)	B15 (759)	Negative
11	Not Known	20%	I		**A19* (2051) B40 (1102)**	Negative
12	Transfusion	31%	II		**DQ7(3)* (1401)**	Negative
13	Transfusion	75%	II	DQA1*01:01 (1743-IgG1) DQA1*01:03 (2210-IgG1)	**DQA1*01:02* (1942)**	Negative
14	Pregnancy	80%	I and II	A10 (2477-IgG1 + IgG3) **DQ7(3)* (4084-IgG1)** DR18(3) (616-IgG3)	A9 (1757) A19 (3117) B13 (1455) B15 (2429) B27 (2318) B37 (1862) B16 (1129) B12 (1326) B47 (2170) B21(3583) B5 (1374) B53 (2763) B17 (2069) B59 (1339)	Negative
15	Transfusion + Pregnancies	80%	II		**DQ6(1)* (852) DQ5(1) (787)**	Negative
16	Transfusion	82%	II		**DQB1*03:02* (969)**	Negative

17	Transfusion + Pregnancies	95%	II	**DR13* (5988-IgG1) DQ6*(1) (12651-IgG1 + IgG2 + IgG3 + IgG4)** DR1 (2002-IgG1) DR17(3) (2211-IgG1) DR4 (1612-IgG1) DR52 (3616-IgG1) DQ5(1) (15245-IgG1 + IgG2 + IgG3) DQB1*03:03 (3251-IgG1)	DR9 (686) DR11(5) (2022) DR15(2) (867) DR16(2) (2917) DR51 (1511) DQB1*03:02 (1813) DQB1*03:03 (1061) DR12(5) (2317)	DQB1*05:01/DQA1*01:01 (5541) **DQ6(1)* (6198)**
18	Transfusion + Pregnancies	83%	II	**DQ7(3)* (8610-IgG1)** DR2 (4033-IgG1)	DR51 (1235)	Negative
19	Previous Transplantation	100%	I and II	**A*30(19) (1539-IgG4)** A3 (1492-IgG4) A11 (2051-IgG4) A10 (7769-IgG1 + IgG2 + IgG3 + IgG4) A31(19) (1813-IgG4) A33(19) (7859-IgG1 + IgG2 + IgG3 + IgG4) A36 (4660-IgG1) A28 (7609-IgG1 + IgG2 + IgG3 + IgG4) A74 (1686-IgG4) B8(4184-IgG1 + IgG4) B*1302 (13) (2640-IgG1 + IgG4) B18 (2044-IgG1 + IgG4) B27 (2058-IgG4) B37 (2896-IgG1 + IgG4) B40 (1731-IgG1 + IgG4) B42 (1523-IgG4) B45 (2035-IgG4) B*5001(21) (2392-IgG4) B*5401(22) (1936-IgG4) B*5601(22) (7265-IgG1 + IgG2 + IgG4) B59 (1154-IgG4) B67 (827-IgG4) B82 (6233-IgG4) DR8 (4812-IgG3) DQ4 (7257-IgG1 + IgG2 + IgG3 + IgG4) DQB1*03:01 (7076-IgG1 + IgG2 + IgG3 + IgG4) DQB1*02:01/DQA1*05:01 (12111- IgG1 + IgG2 + IgG3 + IgG4) DQB1*02:01/DQA1*04:01 (20509 (IgG1 + IgG2 + IgG4) DQB1*05:01/DQA1* 01:01(17565-IgG1 + IgG2 + IgG3 + IgG4) DQB1*05:02/DQA1*01:02 (10329-IgG1)	A1 (1464) A29(19) (1788) A32(19) (827) A43 (977) B15 (2653) B70 (704) B12 (748) B48 (708) B*4901(21) (1251) B*5501(22) (5407) B*1301 (13) (1057) DR1 (901) DR3 (2360) DR9 (929) DR11 (1188) DR13 (951) DR14 (963) DR15(2) (661) DR16(2) (608) DR4 (675) DR12(5) (3350) DR52 (1179) DR53 (772) DR51 (741) **DQB1*02:02/DQA1*0201 (4367)** DQB1*02:01(DQA1*02:01-DQA1*03:01) (2340) DQ7(3) (5370) DQ6(1) (1396)	A10 (4115) A33(19) (5903) A36 (3336) A28 (7950) B8 (706) B37 (843) B*4002(40) (1102) B22 (1821) DQB1*02:01/DQA1*05:01 (11087) DQB1*02:01/ DQA1*04:01 (5442) DQ4 (5677) DQB1*03:01 (12259) DQB1*05:01/DQA1*01:01 (776)
20	Transfusion + Pregnancies	100%	I and II	**A2* (8999-IgG1 + IgG2) B17* (4805-IgG1)** A9 (2592-IgG1) A28 (3166-IgG1) B12 (2059-IgG1) B45 (2979-IgG1) DR1 (15463-IgG1) DR3 (5603-IgG1) DR4 (12401-IgG1 + IgG2) DR9 (11137-IgG1 + IgG2) DR10 (12200-IgG1) DR11(5) (6668-IgG1) DR13(6) (7063-IgG1) DR15(2) (18684-IgG1 + IgG2 + IgG3) DR16(2) (15346- IgG1 + IgG2) **DR51* (13992-IgG1 + IgG2) DR52* (6935-IgG1) DQ6(1)* (2549-IgG1) DQ5(1)* (1885-IgG1)**	B15 (2109) B46 (1455) B73 (2153) DR12(5) (3335) DR14(6) (3377) DQ7(3) (1135)	Negative
21	Previous Transplantation	94%	I and II	**B39* (5347-IgG2)** B7 (3709-IgG2) B8 (5913-IgG2) B15 (4883-IgG2) B70 (5021-IgG2) B18 (4667-IgG2) B27 (6772-IgG2) B35 (5066-IgG2) B39 (5347-IgG2) B40 (3001-IgG2) B45 (5637-IgG2) B48 (3570-IgG2) B21 (5456-IgG2) B67 (4295-IgG2) B81 (4532-IgG2)	B14 (3892) B16 (1784) B42 (5984) B46 (1857) B22 (4969) B73 (2701) B78 (4146) B82 (3167) **DR9* (628)** DR12(5) (608) **DQA1*01:01/01:02/01:03* (765)**	Negative

HLA antibodies are shown in serologic groups; *Anti-HLA donor specific antibodies are highlighted. HLA, human leucocyte antigen; MFI , mean fluoresce intensity; IgGt, immunoglobulin total.

continued to present during the posttransplant period. Four patients presented C1q-binding during posttransplant monitoring, and 2 of these presented pretransplant DSA, whereas 2 formed posttransplant DSA (Figure 2). Four patients presented antibody-mediated rejection (AMR), 3 presensitized and 1 with de novo DSA. T cell–mediated rejection (TCMR) occurred in 2 patients, both with de novo DSA. Two patients lost their allograft, 1 with preformed DSA, and 1 with de novo DSA. Three patients died during the study, 2 with functioning allografts and 1 who developed DSA, C1q-binding, and AMR.

MFI of IgG total and C1q-binding According to IgG Subclass Groups

We analyzed 1598 beads with positive reactions in IgG total for anti-HLA class I, and 2087 positive reactions for anti-HLA class II antibodies. In 630 (39.4%) of class I beads, and 899 (43.1%) of class II beads with low MFI, it was not possible to define the IgG subclass. IgG1 was the predominant IgG subclass found either alone or in combinations with other subclasses; 811 (50.8%) for class I and 1091 (52.3%) for class II antibodies. C1q-binding reactions showed a higher MFI when IgG1 and IgG3 were positive (Table 2).

Pretransplant IgG Subclass Pattern, C1q Reactivity and Previous Sensitization

Twenty-one patients had preformed DSA. Pretransplant antibody profile and sensitization are shown in Table 3. Previous sensitization information was not available for 2 patients. Patients with only transfusions or only pregnancies presented IgG1 and/or IgG3 with lower MFI, whereas patients with both transfusion and pregnancy or previous transplant presented a broader antibody profile with a higher MFI ($P < 0.00001$) (Table 4). 90.7% of the positive beads in previously transplanted patients had IgG2 and IgG4 in its composition and 64.3% of the positive bead reactions in patients with pregnancy plus transfusion presented IgG1 and IgG3 (χ^2=97.504; $P < 0.0001$). C1q-binding was detected in 6 pretransplant patients, of these, 2 were DSA. Patients with C1q-binding antibodies were sensitized by transfusions and pregnancy or previous transplantation.

Posttransplant Changes in IgG Subclass Patterns and C1q Reactivity

Presensitized Patients

Table 5 shows posttransplant evolution of preformed DSA for each patient with the changes in DSA IgG subclasses, ability to bind C1q, allograft function, and clinical events over time during follow-up. Patients 2, 6 and 17 had preformed C1q-binding DSA; but only patients 2 and 17 remained positive after transplantation. Patient 17 presented AMR with minimal (10%) C4d deposition. Patients 4 and 20 were presensitized after multiple transfusions and pregnancies and developed posttransplant C1q-binding DSA showing an increase in MFI values and changes in IgG subclasses patterns, C4d deposition, and AMR. Patient 20 lost the allograft before completing 1 year of transplant.

De Novo DSA Patients

Table 6 shows the posttransplant follow-up for patients that developed DSA after transplantation, with the changes

TABLE 4.
Groups of anti-HLA antibodies IgG subclasses found in pretransplant sera according with previous sensitization

Sensitization cause		Groups of IgG subclasses on pretransplant sera										
		No subclass	IgG1	IgG2	IgG3	IgG4	IgG1 + IgG2	IgG1 + IgG3	IgG1 + IgG4	IgG2 + IgG4	IgG1 + IgG2 + IgG3	IgG1 + IgG2 + IgG4	IgG1 + IgG2 + IgG3 + IgG4
Not known	No. Beads	6	5				1						
	Mean MFI, IgGt	1577	2844				1851						
	SD MFI IgGt	±536.4	±968										
Transfusion	number Beads	45	10		4			3					
	mean MFI IgGt	1362	2937		1234			3400					
	SD MFI IgGt	±825.2	±989.4		±366			±783.6					
Pregnancies	No. Beads	24	6		1			1					
	Mean MFI IgGt	2258	4184		616			2477					
	SD MFI IgGt	±1080.3	±2019.9										
Transfusion + Pregnancy	No. beads	68	78			5	40	11	4		10	1	6
	mean MFI IgGt	1735	5919			1127	7655	2346	3271		9425	10609	12149
	SD MFI IgGt	±1098.3	±3967.5			±473.8	±4404.1	±1533.5	±1947.8		±4846.3		±2102.0
Previous Transplantation	No. beads	70	6	16	1	17			12	1	1	15	17
	mean MFI IgGt	2184	7320	4523	4812	2051			7535	9364	6177	9119	8045
	SD MFI IgGt	±2646.7	±3953.2	±1303.6		±1208,1			±4714.3			±4651.7	±3571.9

Number of all beads with mean fluorescence intensity values over 500 were included in this analysis.

TABLE 5.

Posttransplant antibody monitoring results of presensitized patients

Patient ID	Induction Therapy	Number days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
1	T	0	DQ7(3) (2934-IgG1)	Negative				
		5	DQ7(3) (1148-IgG1)	Negative	44	P+		
		16	DQ7(3) (1290-IgG1)	Negative	74			
		30	DQ7(3) (930-IgG1)	Negative	82			
		76	DQ7(3) (2297-IgG1)	Negative	69			
		94	DQ7(3) (1362-IgG1)	Negative	68			
		184	DQ7(3) (878-IgG1)	Negative	54			
		549	DQ7(3) (2297-IgG1)	Negative	75			
		757	DQ7(3) (891-IgG1)	Negative	73			
2	N	0	B*44:02 (2121-IgG1)	B*44:02 (3083)				
		3	B*44:02 (3111-IgG1)	B*44:02 (7116)	4	P++++		
		16	B*44:02 (5958-IgG1)	B*44:02 (3477)	33			
		27	B*44:02 (6960-IgG1)	B*44:02 (6680)	68			
		90	B*44:02 (3475-IgG1)	B*44:02 (1063)	70	P++		
		164	B*44:02 (5035-IgG1)	B*44:02 (1058)	68			
		369	B*44:02 (8892-IgG1)	Negative	60			
		540	B*44:02 (2102-IgG1)	Negative	79		Negative	No rejection
		842	B*44:02 (1678-IgG1)	Negative	44			
		1266	B*44:02 (3334-IgG1)	B*44:02 (1084)	47			
		1432	B*44:02 (4029-IgG1)	Negative	66			
3	P	0	DR9 (4547) DR53 (4717-IgG3)	Negative				
		3	DR9 (1266)	Negative	14	P+		
		8	DR9 (1742-IgG1) DR53 (1138)	Negative	76	P+		
		13	DR9 (1647-IgG1) DR53 (10231-IgG12)	Negative	65	P+		
		38	DR53 (9607-IgG12)	Negative	74	P+		
		90	DR53 (7348-IgG12)	Negative	59			
		742	DR9 (756) DR53 (4174)	Negative	56			

Continued next page

TABLE 5. (Continued)

Patient ID	Induction Therapy	Number days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
4	N	0	A*24:02 (1847-IgG1) A*26:01 (695) B*08:01 (1269) DRB1*03:01 (9329-IgG12) DRB1*13:01 (9111-IgG12) DR52 (3827-IgG124)	Negative				
		3	Negative	Negative	61			
		7	A*24:02 (8396-IgG123) A*26:01 (7633-IgG1) B*08:01 (5418-IgG123) B*38:01 (10487-IgG12) DRB1*03:01 (5101-IgG12) DRB1*13:01 (11961-IgG12) DR52 (10547-IgG124) DQ2 (8770-IgG1234)	A*24:02 (15962) B*08:01 (15495) DRB1*03:01 (16216) DRB1*13:01 (5928) DR52 (4135)	24	P++		
		11	A*24:02 (5726-IgG1234) A*26:01 (7749-IgG1234) B*08:01 (4799-IgG123) B*38:01 (12140-IgG123) DRB1*03:01 (6852-IgG123) DRB1*13:01 (14181-IgG123) DR52 (12924-IgG1234) DQ2 (13217-IgG1234)	A*24:02 (11306) A*26:01 (7682) B*08:01 (11467) B*38:01 (7437) DRB1*03:01 (16344) DRB1*13:01 (11553) DR52 (10752) DQ2 (7436)	12	P++		
		30	A*24:02 (7919-IgG1234) A*26:01 (2380-IgG13) B*08:01 (9730-IgG123) B*38:01 (2537) DRB1*03:01 (9382- IgG12) DRB1*13:01 (7822-IgG12) DR52 (7161-IgG14) DQ2 (11639-IgG1234)	A*24:02 (12165) DRB1*03:01 (6995) DQ2 (1178)	4	P++++		
		218	A*24:02 (1664) DRB1*03:01 (1269) DR52 (1263) DQ2 (1139)	Negative	17	P+++	C4d+++	AMR (cg0; ci1; ct1; cv1; ah1; mm0)
		390	DRB1*03:01 (781) DR52 (1020) DQ2 (824)	Negative	30	P+		
		745	Negative	Negative	40	P+		
5	OKT3	0	DRB4*01:01 (12416-IgG14) DRB1*04:05 (9364-IgG24)	Negative				
		15	DRB4*01:01 (9998-IgG14) DRB1*04:05 (1798-IgG4)	Negative	70			
		20	DRB4*01:01 (8793-IgG14) DRB1*04:05 (1427-IgG4)	Negative	95			
		28	DRB4*01:01 (8427-IgG14) DRB1*04:05 (688-IgG4)	Negative	94			
		52	DRB4*01:01 (13247-IgG4) DRB1*04:05 (1614-IgG4)	Negative	72			
		92	DRB4*01:01 (9069-IgG4) DRB1*04:05 (1001-IgG4)	Negative	69			
		210	DRB4*01:01 (7985-IgG4) DRB1*04:05 (500-IgG4)	Negative	68			
		368	DRB4*01:01 (7717-IgG4) DRB1*04:05 (727-IgG4)	Negative	70			
		509	DRB4*01:01 (9913-IgG4) DRB1*04:05 (650-IgG4)	Negative	59	P+		
		754	DRB4*01:01 (8746-IgG4) DRB1*04:05 (545)	Negative	72			

6	N	0	A*01:01 (3502-IgG1) B*39:01 (1866-IgG1)	A*01:01 (5011)				
		3	A*01:01 (5304-IgG1) B*39:01 (859)	Negative	5			
		5	A*01:01 (2061-IgG1)	Negative	3	P++		
		30	A*01:01 (4736-IgG1) DQA1*01:02 (1979)	Negative	19	P++		
		61	A*01:01 (7846-IgG1) B*39:01 (1591) DQA1*01:02 (1952-IgG2)	Negative	50	P+		
		89	A*01:01 (3680-IgG1) B*39:01 (862) DQA1*01:02 (1493-IgG2)	Negative	33	P++		
		190	A*01:01 (5022-IgG1)	Negative		P+		
		562	A*01:01 (3016-IgG1)	Negative	47	P+	Negative	No Rejection
		761	A*01:01 (4178-IgG1)	Negative	56	P+		
7	N	0	B*07:02 (3998 -I gG1)	Negative				
		3	Negative	Negative	23			
		10	B*07:02 (1499-IgG1)	Negative	25	P+		
		20	Negative	Negative	35			
		23	B*07:02 (924-IgG1)	Negative	36			
		27	B*07:02 (726-IgG1)	Negative	36			
		1852	Negative	Negative	55			
8	T	0	A*68:02 (5080-IgG12)	Negative				
		5	Negative	Negative	35			
		14	Negative	Negative	45			
		18	Negative	Negative	43			
		20	Negative	Negative	61			
		35	Negative	Negative	53			
		60	Negative	Negative	60			
		125	Negative	Negative	53			
		938	Negative	Negative	32			
		1048	Negative	Negative	22			
		1227	Negative	Negative	17			
9	N	0	B*13:02 (665)	Negative				
		3	Negative	Negative	72			
		10	B*13:02 (1022)	Negative	102			
		23	Negative	Negative	109			
		79	Negative	Negative	85			
		362	Negative	Negative	58			
		708	Negative	Negative	76			

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TABLE 5. (Continued)

Patient ID	Induction Therapy	Number days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
10	T	0	A*01:01 (3689-IgG13)	Negative				
		6	A*01:01 (11681-IgG13)	Negative	53			
		10	A*01:01 (5370-IgG13)	Negative	66			
		19	A*01:01 (5481 -IgG13)	Negative	66			
		29	A*01:01 (2710-IgG13)	Negative	51			
		69	A*01:01 (3688-IgG13)	Negative	64	P+		
		138	A*01:01 (2829-IgG13)	Negative	41			
		208	A*01:01 (897-IgG3)	Negative	49			
11	T	0	A*31:01 (2228)	Negative				
		10	A*31:01 (5571-IgG1)	Negative	27			
		20	A*31:01 (2739-IgG1)	Negative	109			
		63	A*31:01 (1630)	Negative	98			
		90	A*31:01 (1814)	Negative	97			
		176	A*31:01 (1503)	Negative	51			
		358	A*31:01 (1479)	Negative	79			
		601	A*31:01 (1500)	Negative	93			
		772	A*31:01 (1166)	Negative	93			
12	N	0	DQB1*03:01 (3278)	Negative				
		5	DQB1*03:01 (1380)	Negative	33			
		12	DQB1*03:01 (1058)	Negative	45			
		29	Negative	Negative	61			
		56	Negative	Negative	56			
		92	Negative	Negative	60			
		196	Negative	Negative	57			
		390	Negative	Negative	52			
		548	Negative	Negative	65			
13	P	0	DQB1*06:09/DQA1*01:02 (2706-IgG1)	Negative				
		11	DQB1*06:09/DQA1*01:02 (1298)	Negative	4			
		15	DQB1*06:09/DQA1*01:02 (3797)	Negative	12			
		20	DQB1*06:09/DQA1*01:02 (6663-IgG13)	Negative	12			
		30	DQB1*06:09/DQA1*01:02 (5989-IgG13)	Negative	12			
14	I	0	B*37:01 (1862) DQB1*03:01 (4396-IgG1)	Negative				
		5	B*37:01 (1110) DQB1*03:01 (3724-IgG1)	Negative	54			
		16	DQB1*03:01 (4120-IgG1)	Negative	54			
		20	DQB1*03:01 (5951-IgG1)	Negative	62			
		35	DQB1*03:01 (2189-IgG1)	Negative	51			
		63	DQB1*03:01 (912-IgG1)	Negative	46			
		180	DQB1*03:01 (4952-IgG1)	Negative	51			
		365	DQB1*03:01 (1666-IgG1)	Negative	57			

15	P	0	DQB1*06:03/DQA1*01:03 (1002)	Negative				
		10	B*51:01 (1017) DQB1*06:03/ DQA1*01:03 (2390-IgG1)	Negative	74			
		15	B*51:01 (1168) DQB1*06:03/ DQA1*01:03 (1838-IgG1)	Negative	74			
		64	Negative	Negative	57			
		88	Negative	Negative	60			
		730	Negative	Negative	46			
16	N	0	DQB1*03:02 (1527)	Negative				
		5	DQB1*03:02 (1713)	Negative	8			
		15	DQB1*03:02 (1606)	Negative	43			
		30	DQB1*03:02 (1645)	Negative	62			
		92	DQB1*03:02 (3602)	Negative	86			
		142	DQB1*03:02 (2086)	Negative	26		Negative	Acute Pyelonephritis (i1; t2; v0; g0; cv1; cg0; mm0; ah0; ptc0)
		180	DQB1*03:02 (2794)	Negative	18	P+		
		600	DQB1*03:02 (1503)	Negative	34	P++		
17	T	0	DRB1*13:01 (5635-IgG1) DQA1*01:02 (13658-IgG1234)	DQA1*01:02 (5624)				
		17	DRB1*13:01 (11917-IgG123) DQA1*01:02 (6413-IgG12)	DRB1*13:01 (9888) DQA1*01:02 (12744)	42	P++++		
		21	DRB1*13:01 (13771-IgG1234) DQA1*01:02 (7283-IgG123)	DRB1*13:01 (11436) DQA1*01:02 (16646)	51	P++		
		626			27	P+	C4d+	AMR (i2; t1; v0; g2; cv0; cg2; mm1; ah0; ptc2)
18	T	0	DQB1*03:01 (9486-IgG1)	Negative				
		3	DQB1*03:01 (5427-IgG1)	Negative	69	P++		
		15	DQB1*03:01 (3453-IgG1)	Negative	55			
		21	DQB1*03:01 (1821-IgG1)	Negative	52			
		83	DQB1*03:01 (2906-IgG1)	Negative	66	P+		
		169	DQB1*03:01 (2417-IgG1)	Negative	79	P+		
		289	DQB1*03:01 (1367-IgG1)	Negative	55			
		362	DQB1*03:01 (1386-IgG1)	Negative	48			
		519	DQB1*03:01 (1489-IgG1)	Negative	49		Negative	No rejection

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TABLE 5. (Continued)

Patient ID	Induction Therapy	Number days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
19	T	0	A*30:02 (723) A*32:01 (827) B*44:02 (808) B*49:01 (1251) DQA1*02:01 (6773-IgG124)	Negative				
		3	B*49:01 (1101) DQA1*02:01 (6060-IgG124)	Negative	37			
		6	DQA1*02:01 (3874-IgG124)	Negative	45			
		10	DQA1*02:01 (3606-IgG124)	Negative	46			
		17	DQA1*02:01 (6811-IgG124)	Negative	48			
		27	DQA1*02:01 (3839-IgG124)	Negative	48			
		35	DQA1*02:01 (4880-IgG124)	Negative	40			
		60	DQA1*02:01 (4220-IgG124)	Negative	29			
		137	DQA1*02:01 (5331-IgG124)	Negative	45			
		272	DQA1*02:01 (6806-IgG124)	Negative	57		Negative	No Rejection
		553	DQA1*02:01 (5996-IgG124)	Negative	57	P+		
20	T	0	A*02:01 (6635-IgG123) B*57:01 (4483-IgG1) DRB1*16:01 (15645-IgG12) DRB1*11:04 (6595-IgG1) DQB1*03:01 (1372) DQA1*01:02 (2282-IgG1) DRB3*02:02 (7956-IgG1) DRB5*02:02 (17446-IgG12)	Negative				
		5	A*02:01 (3059-IgG123) DRB1*16:01 (5639-IgG1) DRB1*11:04 (640) DQB1*03:01 (2245-IgG1) DQA1*01:02 (1549-IgG1) DRB3*02:02 (904-IgG1) DRB5*02:02 (6902-IgG1)	Negative	56			
		20	A*02:01 (9031-IgG123) B*57:01 (6911-IgG123) DRB1*16:01 (14525-IgG12) DQB1*03:01 (10766-IgG123) DQA1*01:02 (12558-IgG12) DRB3*02:02 (6181-IgG1) DRB5*02:02 (11126-IgG123)	A*02:01 (7179) B*57:01 (1861) DRB1*16:01 (1534) DQA1*01:02 (754) DRB5*02:02 (12402)	6	P+		

30	A*02:01 (10933-IgG123) B*57:01 (6616-IgG123) DRB1*16:01 (11727-IgG13) DRB1*11:04 (5157-IgG1) DQB1*03:01 (8207-IgG123) DQA1*01:02 (8923-IgG12) DRB3*02:02 (4754-IgG1) DRB5*02:02 (7161-IgG123)	A*02:01 (17486) B*57:01 (14806) DRB1*16:01 (3005) DQA1*01:02 (3203) DRB5*02:02 (14071)	6	P+		
75	A*02:01 (3403-IgG123) DRB1*16:01 (9213-IgG123) DRB1*11:04 (2694-IgG1) DQB1*03:01 (5696-IgG123) DQA1*01:02 (4533-IgG123) DRB3*02:02 (3442-IgG1) DRB5*02:02 (1926-IgG123)	A*02:01 (1044) DRB1*16:01 (6240) DQA1*01:02 (16002) DRB5*02:02 (17436)	10	P++		
90	A*02:01 (7201-IgG123) B*57:01 (1073) DRB1*16:01 (14792-IgG123) DRB1*11:04 (5106-IgG1) DQB1*03:01 (12126-IgG123) DQA1*01:02 (14310-IgG12) DRB3*02:02 (6660-IgG1) DRB5*02:02 (13504-IgG123)	A*02:01 (7201) DRB1*16:01 (4935) DQB1*03:01 (14664) DQA1*01:02 (15387) DRB5*02:02 (15590)	12	P+++	C4d+++	AMR (i2; t2; v0; g1; cv0; cg0; mm0; ah0; ptc3)
191	A*02:01 (1958-IgG123) DRB1*16:01 (12159-IgG123) DRB1*11:04 (1040) DQB1*03:01 (4805-IgG123) DQA1*01:02 (6732-IgG123) DRB3*02:02 (2457-IgG1) DRB5*02:02 (6732-IgG123)	DRB1*16:01 (4090) DQB1*03:01 (16545) DQA1*01:02 (14796) DRB5*02:02 (8203)	12	P++	C4d+++	AMR (i2; t1; v0; g2; cv0; cg1; mm1; ah0; ptc2)
237	A*02:01 (3845-IgG123) DRB1*16:01 (13954-IgG13) DRB1*11:04 (1968) DQB1*03:01 (6511-IgG123) DQA1*01:02 (6527-IgG123) DRB3*02:02 (3604) DRB5*02:02 (6630-IgG1)	Not tested	12	P++		Allograft Loss

Continued next page

TABLE 5. (Continued)

Patient ID	Induction Therapy	Number days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
21	T	0	B*39:01 (5347-IgG12)	Negative				
		5	Negative	Negative	48			
		10	B*39:01 (4382-IgG1) DRB1*09:01 (1318-IgG1) DRB1*14:01 (974-IgG1)	Negative	50			
		19	B*39:01 (4791-IgG1234) DRB1*09:01 (1318-IgG1) DRB1*14:01 (974-IgG1)	Negative	34			
		390	Negative	Negative	50			
		416	Negative	Negative	50			
		751	Negative	Negative	43			

Induction therapy: N, none; T, thymoglobulin; I, anti-IL2mAb; P, prednisone; OKT3, MuronAb-CD3. Proteinuria: P+, 150-500 mg; P++, 500-1000 mg; P+++, 1000-3500 mg; P++++, >3500 mg. C4d: C4d+, minimal deposition (<10%); C4d++, focal deposition (10-50%); C4d+++, diffuse (>50%).

of IgG subclasses, ability to bind C1q, allograft function, and clinical events. Patients 22 and 25 formed C1q-binding DSA. The first anti-HLA DSA detected in patient number 22 was on day 90 after transplant with no C1q binding, followed by an increase in MFI and change in the subclass profile from IgG1 and/or IgG3 to all subclasses and C1q-binding. This patient developed AMR and lost the allograft before completing 2 years of transplant.

C1q Reactivity and Allograft Function and Survival

Allograft function over time was evaluated by comparing the mean eGFR MDRD by period (Figure 3A) and by ANOVA repeated measurements (Figure 3B). Patients with C1q binding anti-HLA DSA showed lower allograft function from the first year of transplant through the fifth year. Allograft loss was only observed in patients with C1q binding anti-HLA DSA (100% survival rate at 5 years for DSA+C1q– patients compared to 71.4% survival rate for patients DSA+C1q+).

DISCUSSION

In the present study, we sequentially followed up 30 patients with anti-HLA DSA (mean of 12 samples per patient) to evaluate characteristics such as HLA class, IgG subclass, C1q-binding ability, changes in reaction patterns over time after transplant, time of AMR, TCMR, and allograft dysfunction. All transplants required a negative T and B cell CDC-XM at the time of transplantation. The presence of DSA of any MFI value was not a counter indication for transplantation.

In pretransplant and posttransplant sera, IgG1 was the most common IgG subclass. Presensitized patients showed different compositions of IgG subclasses according to the cause of sensitization. Patients sensitized by only transfusion or pregnancies had anti-HLA antibodies of IgG1 and/or IgG3 subclasses, whereas patients with both transfusion and pregnancies and previous transplant showed a broader range of IgG subclasses. This is in accordance with data presented by Lowe et al.[19]

We found IgG3 more frequently than IgG2 in posttransplant sera rather than the expected order of the IgG subclass concentration IgG1, IgG2, IgG3, and IgG4.[23] The evaluation of sequential sera allowed for detection of subclasses at different stages of the immune response. We hypothesize that like other diseases, such as membranous glomerulonephritis,[24,25] development of AMR and its progression is related to subclass switching. There were also differences between patients with preformed DSA and de novo DSA, in which de novo DSA were primarily made up of IgG1 and IgG3 alone. Only 1 patient with de novo DSA presented AMR, and subsequently, allograft dysfunction. The first IgG detected was a class II DSA of IgG3 subclass with no C1q-binding ability in vitro. We subsequently detected all subclasses and C1q binding in the next serum, which presented AMR features in the tissue with minimal C4d deposition. It has been suggested that subclass switching occurs first from IgM to IgG3 and then to IgG1, IgG2, and IgG4. In many responses early antigen clearance would prevent the appearance of IgG2 and IgG4.[12] IgG2 and IgG4 were detected, but only in combination with other subclasses, demonstrating an evolution of the immune response. The presence of IgG2 and IgG4 was shown in elutes of rejected renal allografts confirming sequential subclass switching.[16] Moreover,

TABLE 6.

Posttransplant antibody monitoring results of de novo DSA patients

Patient ID	No. days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
22	3	0	0	45			
	5	0	0	66			
	15	0	0	57			
	27	0	0	76			
	30	0	0	87			
	62	DQB1*03:01 (IgM)	0	53	P++		
	90	DR8 (1422-IgG3) DQB1*03:01 (790)	0	65	P++		
	182	B35 (IgM) B53 (IgM) DR8 (1978-IgG3) DRB1*03:01 (2200-IgG13)	DQB1*03:01 (3020)	59	P++	C4d+	AMR (i2; t2; v0; g1; cv0; cg0; mm0; ah0; ptc1)
	432	A1 (869-IgG13) A36 (339-IgG13) DR8 (1541-IgG3) DQB1*03:01 (2546-IgG1234) DQA1*04:01 (2260-IgG1234)	A36 (627) DQB1*03:01 (11275) DQA1*04:01 (11138)	64	P++		
	687	A1 (4164-IgG134) A36 (4498-IgG1234) B53 (820) DR8 (3373-IgG3) DQB1*03:01 (2989-IgG1234) DQA1*04:01 (9983-IgG1234)	A1 (4886) A36 (4131) DQB1*03:01 (8829) DQA1*04:01 (9543)	37	P++		Allograft Loss
23	10	0	0	62			
	13	0	0	48			
	20	0	0	44			
	27	0	0	40			
	1832	DQB1*03:03 (2523)	0	70			
24	3	DQ2/DQA1*02:01 (3130)	0	51			
	5	0	0	54	P++		
	12	0	0	71			
	30	0	0	65			
	48	DQ2/DQA1*02:01 (876)	0	31			
	71	0	0	46			
	168	DQ2/DQA1*02:01 (1398)	0	55			
	365	0	0	66			
	580	DQ2/DQA1*02:01 (1071)	0	61			
	760	DQ2/DQA1*02:01 (1190)	0	53			
	1024	0	0	41			
	1189	0	0	43			
	1290	0	0	31			
25	2	0	0	37	P+		
	5	0	0	42	P+		
	8	0	0	59	P+		
	1717	DQ5(1) (7601-IgG1)	DQ5(1) (1276)	71			
26	10	DQB1*02:01/DQA1*05:01 (2477-IgG2)	0	31			
	15	DQB1*02:01/DQA1*05:01 (2916-IgG2)	0	35	P++		
	30	0	0	43			
	34	0	0	39			
	63	0	0	41			
	92	0	0	39			
	187	0	0	43			

Continued next page

TABLE 6. (Continued)

Patient ID	No. days posttransplant	DSA profile	C1q-binding	eGFR MDRD	Proteinuria	C4d	Biopsies (BANFF 2017 score)
27	3	A3 (581)	0	61			
	5	A3 (624)	0	64			
	15	0	0	61			
	30	0	0	44			
	183	0	0	46			
	474	0	0	22			
	489	0	0	27			
	524	0	0	32			
	564	0	0	32			
28	13	0	0	61			
	15	0	0	58			
	18	0	0	53			
	26	0	0	57			
	69	0	0	66			
	94	0	0	67			
	180	0	0	51			
	545	DQA1*05:01/02/03 (2675-IgG13)	0	48			
	736	DQA1*05:01/02/03 (2676-IgG1)	0	90			
29	3	0	0	41			
	5	0	0	32			
	15	0	0	36	P++	Negative	TCMR (IA-i2; t2; v0; g0; cv0; cg0; mm0; ah0; ptc1)
	20	0	0	48	P+		
	30	0	0	55			
	34	DQB1*03:01 (1801-IgG3)	0	55			
	56	DQB1*03:01 (678-IgG3)	0	47			
	90	DQB1*03:01 (568-IgG3)	0	43			
	515	0	0	56			
	730	0	0	66			
30	3	0	0	5			
	6	0	0	7			
	10	0	0	4			
	20	0	0	34			
	22	0	0	46			
	31	0	0	51			
	68	0	0	53			
	171	0	0	50			
	374	0	0	54			
	705	DQA1*03:02 (4707-IgG1)	0	52			TCMR (IFTA discreet; i1; t0; v0; g0; cv0; cg0; mm0; ah0; ptc0)
	1329	DQA1*03:02 (1457-IgG1)	0	51		P++	

IFTA, interstitial fibrosis and tubular atrophy.

there seemed to be a correlation between the occurrence of AMR and the expansion of complement-fixing to noncomplement fixing DSA.[15]

Another important observation was that despite the high prevalence of IgG1 subclass, C1q-binding was found in less than 15% of the sera tested, and only in the presence of high MFI IgG1 or, most frequently, in the presence of a combination of all 4 subclasses. As recently shown by Schaub et al, C1q binding is related to anti-HLA antibody density, and furthermore, a great number of HLA antibodies found in sera that do not induce C1q-binding in vitro do, however, contain C-binding IgG subclasses (IgG1 and IgG3).[11] The presence of all 4 subclasses can indicate a higher antibody concentration, thus providing higher density for C1q-binding. The presence of a high concentration of antibodies sequentially binding to antigens leads to hexamer formation that binds to C1q with higher avidity than monomeric IgG, inducing activation of the complement system.[26]

Antibody-mediated rejection is one of the leading causes of allograft failure.[27] Although the presence of DSA implies risk for AMR, long-term survival of patients with DSA has been reported.[28,29] Despite our small population number, we observed that most patients diagnosed with AMR presented an increase in MFI, changes in IgG subclasses, and C1q-binding DSA. Loupy et al reported that patients with donor specific C1q-binding DSA present lower allograft survival

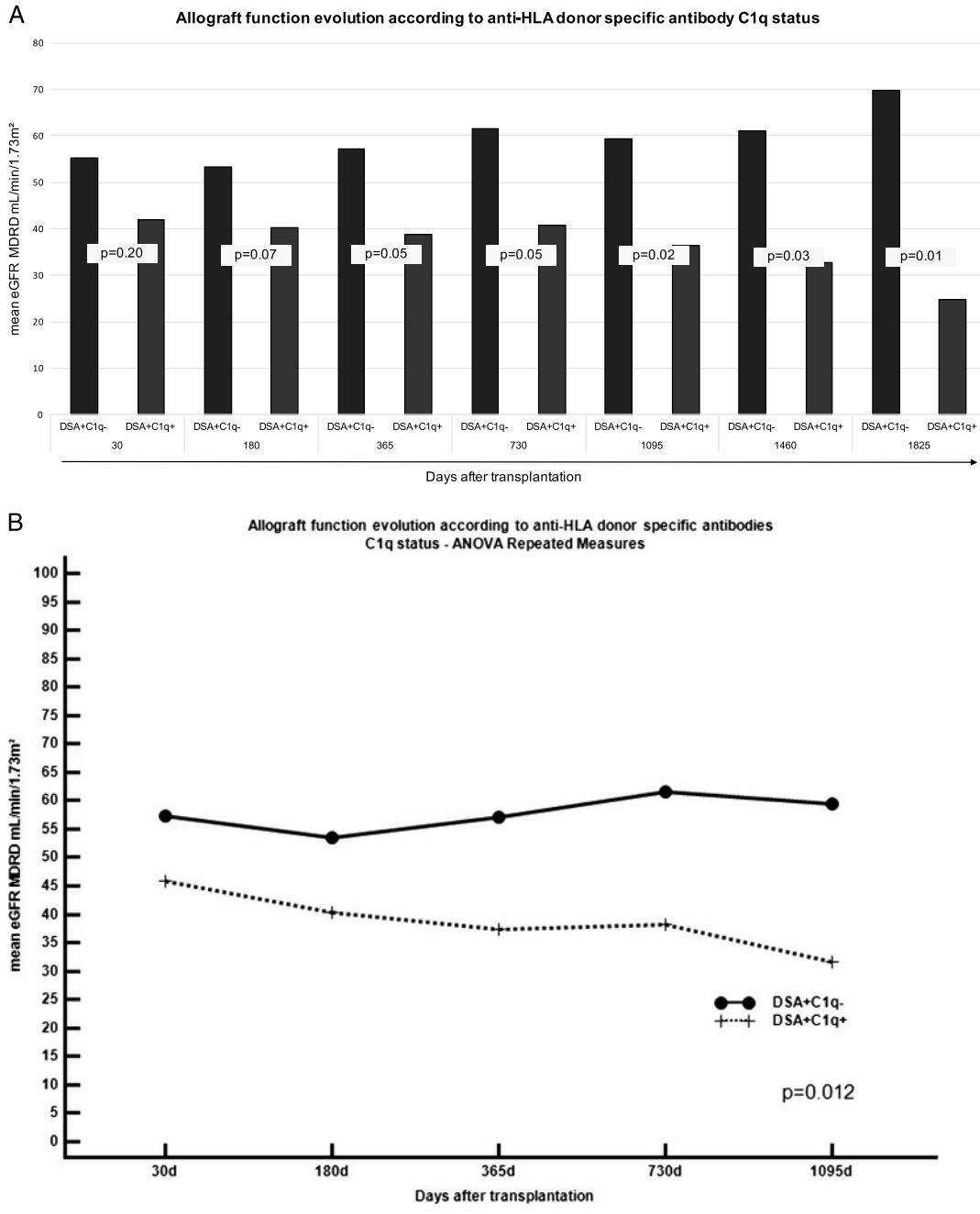

FIGURE 3. Evolution of allograft function. A, Comparison of means of allograft function measured by eGFR by MDRD according to the presence of anti-HLA DSAs with or without C1q-binding by each period of time evaluated. B, Evolution of allograft function over time evaluated by repeated-measures ANOVA according to C1q-binding status.

within 5-years of follow-up when compared with non–complement-binding DSA and non-DSA patients.[10] Moreover, pediatric kidney recipients with de novo C1q DSA reactivity showed higher rates of rejection and increased risk of allograft loss.[30] The presence of a combination of IgG subclasses with C1q-binding DSA could also be related to AMR occurrence, and diminished allograft function. It was previously shown that liver transplant patients with IgG subclass combinations containing IgG3 presented allograft survival that was significantly lower than patients who presented a single IgG subclass. Changes in the profile of antibodies during posttransplant follow-up demonstrate the importance of close anti-HLA DSA monitoring after transplantation.[31] Moreover, the presence of different subclasses can indicate distinct phenotypes of AMR. IgG4-containing DSA was associated with features of subclinical AMR, whereas IgG3-containing DSA was associated with an acute form of AMR and represented a greater risk for allograft loss.[19]

Our study presents certain limitations, including the small population and testing at different time points with different lots of SAB for DSA. In addition, we were unable to evaluate denatured antigens to confirm the IgG subclass negative reactions.

Recently, 3 different stages of AMR were described by molecular diagnosis of kidney biopsies; early-stage AMR, fully

formed AMR, and late-stage AMR.[32] Understanding the natural evolution of anti-HLA antibodies during the process of AMR and correlating to its stages is essential to define treatment. Although our data are not conclusive, we demonstrate that there is a progression of the immune response and it can begin at the sensitization cause and may consequently lead to allograft loss. These insights should be considered if patients are not consistently monitored for anti-HLA antibodies after transplantation. Thus, a single time point evaluation after transplantation may not be sufficient to provide all the information needed to make clinical decisions.

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