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Detection of donor-specific HLA antibodies before and after removal of a rejected kidney transplant

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ABSTRACT

Serum analysis of patients considered for retransplantation has a potential limitation that the rejected allograft may absorb HLA antibodies. We have determined how the highly sensitive micro bead-based Luminex antibody-binding assay with single antigens can detect donor-specific HLA antibodies (DSA) in patients before and after surgical removal of a rejected allograft. This analysis was done for 65 allograft nephrectomy (allonx) cases contributed by 16 laboratories worldwide.

In the HLA-A,B and -DRB1 mismatch categories the incidence of DSA reactivity pre-allonx and post-allonx was 64% vs 87% ($p=0.0033$) and 57% vs 86% ($p=0.001$), respectively. The frequencies of individual reactive antigens were also lower before allonx: for HLA-A,B antigens: 49% vs 75% ($p<0.0001$) and DRB1 antigens: 48% vs 79% ($p=0.0001$). On the other hand, no significant differences were seen between the pre-allonx and post-allonx frequencies of DSA to DRB3/4/5 (65% vs 78%, $p=0.22$) and DQ mismatches (76% vs 87%, $p=0.18$).

Conclusion: although the sensitive Luminex antibody assay can detect anti-donor antibodies in the presence of a rejected transplant, it is apparent that the antibody specificity pattern is often incomplete especially against the HLA-A, -B and DR mismatches. This understanding seems relevant to the determination of acceptable mismatches for patients considered for retransplantation.

1. Introduction

Antibody responses to donor HLA class I and class II antigens represent significant risk factors for kidney transplant failure [1]. Prior to transplantation, recipient sera are tested for HLA antibodies and a determination of donor mismatch acceptability increases transplant success. After transplantation, the development of donor-specific HLA antibodies leads to acute rejection and chronic rejection. Traditionally, serum testing for HLA antibodies is done with complement-dependent lymphocytotoxicity techniques but during recent years, most laboratories are also using antibody-binding assays that have greater sensitivity. Especially, Luminex assays with single HLA class I and class II allele panels [2] are now widely used for HLA antibody analysis.

HLA antibody testing in transplant recipients has a potential limitation in that certain donor-specific antibodies are undetectable because they have been absorbed by the transplanted organ. This is an important consideration in the determination of HLA mismatch acceptability for patients who have rejected their transplant and are now considered for retransplantation. In support of this notion, many studies have shown the appearance of previously undetectable lymphocytotoxic antibodies following surgical removal of the transplant [3–11]. Moreover, donor-specific HLA antibodies can be eluted from rejected transplants [11–15].

Since the Luminex assay with single HLA alleles on microbeads is considerably more sensitive than lymphocytotoxicity [16,17], we addressed the question how this method permits the detection of donor-specific antibodies in the presence of a rejected kidney transplant. We have analyzed HLA antibody reactivities of sera from patients who have undergone allograft nephrectomy (allonx). This study is part of a multilaboratory collaborative project on HLA epitope immunogenicity and conducted under auspices of the 15th International Histocompatibility and Immunogenetics Workshop. The results show that even the sensitive Luminex assay will often yield a limited detectability of donor-reactive HLA antibodies in the presence of a failed allograft.

2. Materials and methods

2.1. Patients and Sera

This serum analysis was done on 65 allonx cases contributed by 16 laboratories worldwide participating in the 15th International Histocompatibility Workshop project on HLA epitope immunogenicity (Table 1). These participants provided the following information. All

Abbreviations: Allonx, allograft nephrectomy; DSA, donor-specific antibody; HLA, human leukocyte antigen; MFI, median fluorescence intensity.

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Table 1
15th International Histocompatibility Workshop participants who contributed cases with pre- and post-allograft nephrectomy sera.

Participant	Institution	Location	Number of cases
Wil Allebes	University Medical Center St Radboud	Nijmegen, The Netherlands	2
Patricia Campbell	University of Alberta Hospitals	Edmonton, Canada	11
Anne Cesbron Gautier	EFS – Laboratoire HLA	Nantes, France	8
Silvia Chrenova	Slovak Medical University	Bratislava, Slovakia	5
Ilias Doxiadis	Leiden University Medical Center	Leiden, Netherlands	2
Amy Hahn	Albany Medical College	Albany, NY, USA	5
Andres Jaramillo	Gift of Hope Organ & Tissue Network	Elmhurst, NY, USA	1
Ed Kaminski	Derriford Combined Laboratories	Plymouth, UK	2
Tirza Klein	Rabin Medical Center	Petah Tikva, Israel	1
James McCluskey	Australian Red Cross Blood Services	Adelaide, Australia	7
Anne Parissiades	Établissement Français du Sang – Alsace	Strasbourg, France	2
Lorita Rebellato	ECU Brody School of Medicine	Greenville, NC, USA	3
Constanze Schönemann	Univ Clin Charite Campus Virchow Klinikum	Berlin, Germany	2
Agathi Varnavidou	Paraskevaidion Transplant Center	Nicosia, Cyprus	2
Cristina von Glehn	Pontificia Universidade Catolica	Curitiba, Brazil	4
Adriana Zeevi	University of Pittsburgh Medical Center	Pittsburgh, PA USA	8
		Total	65

cases were primary transplant recipients with no evidence of pre-transplant HLA sensitization: PRA < 15% and a negative cross-match. HLA-A, -B, -C, -DR and -DQ typing of patients and donors was performed by the contributing laboratories by standard serological and/or molecular methods. All cases involved at least one HLA-A,-B and/or HLA-DR,-DQ antigen mismatch and 22 (34%) transplants had been done from living donors. Immunosuppressive treatments were according to standard protocols at participant institutions. All 65 cases were failed transplants due to rejection that had been surgically removed 2421 ± 2003 days post-transplant for unspecified clinical reasons and 60 of them (92%) were done after more than one year. Insufficient information was available about rejection types or transplant pathology. Serum specimens were obtained a median of 35 days before allonx (range 1–306 days) and 44 days after allonx (range 14–337 days). Antibody testing was always done on paired pre-allonx and post-allonx sera.

2.2. Serum screening for HLA antibodies

This was done with micro bead Luminex assays using single HLA allele kits supplied by two commercial vendors (One Lambda Inc., Canoga Park, CA; Tepnel Life Codes Corporation, Stamford, CT) according to the manufacturer's instructions. In brief, an aliquot of a mixture of Luminex microspheres, each coated with a single antigen, was incubated with a small volume of test serum sample and washed to remove unbound antibody. Anti-human immunoglobulin antibody conjugated to phycoerythrin was added and after incubation the bead mixture was diluted for analysis with the LABScan 100 instrument (Luminex, Austin, TX) and the reactivity was determined with the manufacturer's software.

2.3. Determination of anti-donor antibody reactivity

The presence of antibody was determined by comparing the median fluorescence intensity (MFI) of the beads containing the individual antigens to the signal intensities of positive and negative controls. We also calculated the average MFI for the patient's self HLA antigens as another negative control. MFI values with HLA antigens in the panel were considered positive if (MFI with antigen minus average MFI with self-antigens)/(MFI with positive control minus average MFI with self-antigens) > 10%. Positive reactions of patient sera with antigens shared between the panel and the transplant donor were considered evidence for the presence of anti-donor antibodies.

2.4. Statistical methods

Differences between the various groups were analyzed for significance with statistical methods including chi-square analysis and student *t*-tests for paired samples or two-samples assuming unequal variances.

3. Results

3.1. Detection of donor-specific HLA class I antibodies

Our analysis of the serum reactivity patterns addressed initially the question whether donor-specific antibodies (DSA) could be detected before and after allonx. This was done by identifying in the single allele panel, which donor HLA antigen mismatch gave the highest MFI value with the pre-allonx serum in comparison with the MFI with the post-allonx serum. Sixty-two cases with HLA-A, B antigen mismatches were available and as shown in Table 2, the reactivity patterns were categorized as (1) no detectable DSA pre- and post-allonx ($N = 8$, 13%), (2) only post-allonx DSA ($N = 14$, 23%) and, (3) both pre- and post-allonx DSA ($N = 40$, 64%). Within the latter group, the pre-allonx sera had significantly lower MFI values for DSA than the post-allonx sera (7959 ± 5190 vs 10251 ± 4877, $p = 0.0015$). These findings show that DSA against HLA-A,B are often detected in pre-allonx sera but that their frequency and strength are significantly lower than in post-allonx sera.

Interestingly, for the 8 cases with no detectable DSA, the post-allonx sera had been collected at shorter time intervals than the 54 cases with DSA (42 ± 20 vs 82 ± 87, $p = 0.006$). There was no association between the incidence of DSA and the number of HLA-A, B antigen mismatches (2.4 ± 1.0 vs 2.8 ± 0.8, $p = 0.3$), but we also noted that for the 8 DSA-negative cases, the allonx was done on a later post-transplant day than the 54 cases with DSA (4121 ± 1838 vs 2219 ± 1710, $p = 0.03$).

3.2. Antibody reactivity with donor HLA class I antigens

These 62 class I mismatched cases had a total of 167 donor HLA-A,B antigen mismatches and Table 3 summarizes the incidence of DSA against them before and after

Table 2
DSA reactivity agianst HLA-A,B mismatches in pre-allonx and post-allonx sera.

DSA pre and post-allonx	Number of cases	Antigen mismatches	Allonx (days)	Pre-allonx serum (days)	MFI with self HLA	MFI positive control	MFI-dab pre-allonx	Post-allonx Serum (days)	MFI with self HLA	MFI positive control	MFI-dab post-allonx
(1) Negative, negative	8 (13%)	2.4 ± 1.0	4121 ± 1838[a]	66 ± 78	204 ± 110	11656 ± 2272	366 ± 310	42 ± 20[b]	193 ± 112	11170 ± 2651	540 ± 560
(2) Negative, positive	14 (23%)	2.8 ± 1.1	1194 ± 985	86 ± 93	235 ± 191	11288 ± 3635	441 ± 325	136 ± 117	212 ± 126	10288 ± 4015	9315 ± 4344
(3) Positive, positive	40 (64%)	2.8 ± 0.7	2569 ± 1774	62 ± 69	487 ± 385	11083 ± 4471	7959 ± 5190[c]	64 ± 64	529 ± 639	10813 ± 4241	10251 ± 4877[c]

[a] Post-transplant allonx days: (1) versus (2) + (3), $p = 0.03$ (two-tail *t*-test assuming unequal variances).
[b] Post-allonx serum days: (1) versus (2) + (3), $p = 0.006$ (two-tail *t*-test assuming unequal variances).
[c] Pre-allonx versus post-allonx MFI values in (3): $p = 0.0015$ (paired *t*-test).

Table 3
Frequencies of DSA reacting with individual class I antigens in pre-allonx and post-allonx sera.

Mismatched antigen	Nr of cases	Positive pre-allonx	Positive post-allonx
HLA-A1	12	58%	75%
HLA-A2	18	89%	89%
HLA-A3	8	38%	75%
HLA-A24	10	40%	70%
HLA-A26	7	71%	100%
HLA-A32	7	14%	43%
Other HLA-A antigens	19	53%	95%
All HLA-A antigens	**81**	**57%**[a]	**81%**[a]
HLA-B7	8	75%	88%
HLA-B8	9	56%	78%
HLA-B27	12	42%	75%
HLA-B35	9	44%	89%
HLA-B16 (B38 or B39)	7	0%	29%
HLA-B44	8	50%	100%
HLA-B5 (B51 or B52)	6	50%	67%
Other HLA-B antigens	27	30%	52%
All HLA-B antigens	**86**	**41%**[b]	**69%**[b]
HLA-Cw2	6	33%	33%
HLA-Cw3	7	43%	29%
HLA-Cw7	6	50%	50%
Other HLA-C antigens	14	14%	29%
All HLA-C antigens	**33**	**30%**[c]	**33%**[c]

[a] $p=0.0007$.
[b] $p=0.0002$.
[c] $p=0.79$ (not significant).

allonx. Individual antigens are listed if at least 6 cases were available. Donor antigen reactivity with serum was considered positive if the adjusted MFI was >10% of the adjusted positive control. Except for the highly reactive HLA-A2, all HLA-A and HLA-B antigens listed in Table 3 reacted more frequently with post-allonx sera than pre-allonx sera. For the total of 81 HLA-A and 86 HLA-B mismatches, the DSA incidence was 81% vs 57% ($p=0.0007$) and 69% vs 41% ($p=0.0002$), respectively. We noted also a trend towards a higher DSA incidence against HLA-A than HLA-B (pre-allonx: 57% vs 41%, $p=0.045$; post-allonx: 81% vs 69%, $p=0.055$). Altogether, among the 167 HLA-A,B antigen mismatches, 49% reacted with pre-allonx sera but 75% reacted with post-allonx sera ($p<0.00001$).

HLA-C typing information and serum testing with HLA-C typed panels was provided for 26 cases. There were 33 HLA-C antigen mismatches, including Cw2, Cw3 and Cw7 which were mismatched in at least six cases. The incidence of DSA against HLA-C was practically the same in pre-allonx and post-allonx sera (30% vs 33%, $p=0.79$). Moreover, DSA was less common for HLA-C than HLA-A and HLA-B, especially in post-allonx sera (33% vs 75%, $p<0.0001$).

3.3. Detection of donor-specific HLA class II antibodies

There were 53 allonx cases with HLA-DR antigen mismatches and first we determined if any DSA could be detected by Luminex in pre- and post-allonx sera. This analysis considered the conventional HLA-DR antigens as well as DR51, DR52 and DR53. Table 4 shows three reactivity patterns: (1) no detectable DSA pre- and post-allonx ($N=7$, 14%), (2) only post-allonx DSA ($N=13$, 29%) and, (3) both pre- and post-allonx DSA ($N=30$, 57%). Within the latter group, the pre-allonx sera had significantly lower MFI values for DSA than the post-allonx sera (9074 ± 5622 vs 11557 ± 4660, $p=0.0008$). These findings show that DSA against HLA-DRB antigen mismatches are often detected in pre-allonx sera but that their frequency and strength are significantly lower than in post-allonx sera.

A similar analysis of DSA responses to DQ mismatches considered the DQ2, DQ4, DQ5, DQ6, DQ7, DQ8 and DQ9 antigens. As shown in Table 4 there were again three reactivity patterns: (1) no detectable DSA pre- and post-allonx ($N=6$, 13%), (2) only post-allonx DSA ($N=5$, 11%) and, (3) both pre- and post-allonx DSA ($N=36$, 76%). Although the frequencies of DSA in post-allonx sera were similar for DR and DQ (86% and 87%, respectively), these findings show that DQ-specific DSA are more readily detected in pre-allonx sera than DR-specific DSA (76% vs 57%, $p=0.043$). Moreover, in the 36 cases with both pre-allonx and post-allonx DSA reactivity, there were no significant differences between their MFI values (9763 ± 5789 vs 11002 ± 4926, $p=0.13$). These data suggest that anti-DQ DSA are generally readily detected in pre-allonx sera and that an increased detection of these DSA post-allonx is at best, quite small.

As shown in Table 4, a small proportion of class II mismatches showed no DSA (Group (1): 14% for DR and 13% for DQ). In both instances, there was a significant correlation with a low number of mismatched antigens ($p=0.0004$ for DR and $p=0.00001$ for DQ). We also noted that similar to the class I cases (Table 2), the lack of DSA was associated with a later allonx time (DR: $p=0.05$; DQ: $p=0.04$) and perhaps, an earlier post-allonx serum collection day (DR: $p=0.02$; DQ: $p=0.24$, not significant).

3.4. Antibody reactivity with donor HLA class II antigens

These 52 DR mismatched cases had a total of 72 donor DRB1 and 40 donor DRB3,4,5 antigen mismatches. Table 5 summarizes the incidence of DSA reactivity with them before and after allonx. The DRB1 antigens reacted more frequently with post-allonx sera than pre-allonx sera (79% vs 48%, $p=0.0001$). In contrast, DSA against DR51, DR52 and DR52 were slightly higher post-allonx but the difference was not significant (78% vs 65%, $p=0.22$). The 47 DQ mismatch cases involved 57 DQ antigens, but DQ-specific DSA reactivity was not significantly higher post-allonx than pre-allonx (86% vs 72%, $p=0.07$). These findings suggest that DRB1-reactive DSA are less readily detectable in pre-allonx sera than DRB3/4/5-reactive and DQ-reactive DSA.

Table 4
DSA reactivity against HLA-DR and HLA-DQ mismatches in pre-allonx and post-allonx sera.

Anti-donor antibody pre and post-allonx	Number of cases	Antigen mismatches	Allonx (days)	Pre-allonx serum (days)	MFI with self HLA	MFI positive control	MFI-DSA pre-allonx	Post-allonx serum (days)	MFI with self HLA	MFI positive control	MFI-DSA post-allonx
Against HLA-DRB1/3/4/5											
(1) Negative, negative	7(14%)	1.3 ± 0.5[a]	3935 ± 1749[b]	115 ± 150	282 ± 251	9285 ± 3941	252 ± 175	48 ± 20[c]	281 ± 237	8787 ± 4109	404 ± 405
(2) Negative, positive	15 (29%)	2.1 ± 1.0	1084 ± 868	65 ± 67	274 ± 273	10701 ± 3526	442 ± 494	136 ± 113	409 ± 403	10065 ± 3333	6996 ± 4708
(3) Positive, positive	30 (57%)	2.4 ± 0.7	2240 ± 1385	76 ± 85	589 ± 507	9872 ± 4586	9074 ± 5622[d]	66 ± 68	855 ± 1235	11121 ± 3802	11557 ± 4660[d]
Against HLA-DQB											
(1) Negative, negative	6 (13%)	1.0 ± 0.0[e]	3934 ± 1283[f]	93 ± 133	321 ± 252	9235 ± 4310	395 ± 230	53 ± 32[g]	308 ± 269	9268 ± 4933	515 ± 574
(2) Negative, positive	5 (11%)	1.2 ± 0.4	1276 ± 1380	75 ± 101	231 ± 127	9023 ± 4039	790 ± 568	157 ± 127	476 ± 434	11191 ± 3071	7712 ± 5338
(3) Positive, positive	36 (76%)	1.4 ± 0.5	2096 ± 1470	88 ± 95	430 ± 375	9752 ± 4429	9763 ± 5789[h]	75 ± 76	642 ± 1030	10263 ± 4004	11002 ± 4926[h]

[a] (1) vs (2) + (3), $p=0.004$.
[b] (1) vs (2) + (3), $p=0.045$.
[c] (1) vs (2) + (3), $p=0.02$.
[d] paired t-test: $p=0.0008$.
[e] (1) vs (2) + (3), $p=0.00001$.
[f] (1) vs (2) + (3), $p=0.04$.
[g] (1) vs (2) + (3), $p=0.24$ (NS).
[h] paired t-test: $p=0.13$ (NS).

Table 5
Frequencies of DSA reacting with individual class II antigens in pre-allonx and post-allonx sera.

Mismatched antigen	Nr of cases	Pre-allonx	Post-allonx
HLA-DR1	9	33%	67%
HLA-DR4	10	50%	90%
HLA-DR11	8	63%	75%
HLA-DR13	14	50%	64%
HLA-DR15	9	78%	89%
Other HLA-DR	23	31%	85%
All HLA-DRB1	**72**	**48%**[a]	**79%**[a]
HLA-DR51	11	73%	73%
HLA-DR52	19	53%	74%
HLA-DR53	10	80%	90%
All HLA-DRB3/4/5	**40**	**65%**[b]	**78%**[b]
HLA-DQ2	8	63%	88%
HLA-DQ5	10	40%	70%
HLA-DQ6	13	69%	85%
HLA-DQ7	17	94%	94%
Other HLA-DQ	9	78%	89%
All HLA-DQ	**57**	**72%**[c]	**86%**[c]

[a] $p = 0.0001$.
[b] $p = 0.22$ (NS).
[c] $p = 0.07$ (NS).

Table 6
Summary of donor-specific HLA antibody reactivity of sera before and after allograft nephrectomy.

	HLA-A,B,C	HLA-DR	HLA-DQ
Incidence of anti-donor antibodies	64% vs 87% $p = 0.0033$	57% vs 86% $p = 0.001$	76% vs 87% $p = 0.18$ (NS)*
Strength of anti-donor antibodies (MFI values)	7959 ± 5190 vs 10251 ± 4877 $p = 0.0015$	9074 ± 5622 vs 11557 ± 4660 $p = 0.0008$	9763 ± 5789 vs 11002 ± 4926 $p = 0.13$ (NS)
% Reactive donor antigen mismatches	HLA-A,B: 49% vs 75% $p < 0.00001$ HLA-C: 30% vs 33% $p = 0.79$ (NS)	DRB1: 48% vs 79% $p = 0.0001$ DRB3/4/5: 65% vs 78% $p = 0.22$ (NS)	DQB1: 72% vs 86% $p = 0.07$ (NS)

4. Discussion

This analysis addressed the detection of donor-specific HLA antibodies before and after surgical removal of a rejected allograft. In patients considered for retransplantation, the issue of HLA antibody absorbance by the first allograft seems important for the determination of HLA mismatch acceptability from serum antibody reactivity patterns and the identification of suitably matched donors. This study demonstrates that even with a highly sensitive antibody detection assay such as Luminex with single antigens, most patients display incomplete antibody reactivity patterns in the presence of the failed transplant. Table 6 summarizes the incidence and strength of anti-donor antibodies and the percentages of donor HLA antigen mismatches recognized by such antibodies before and allograft nephrectomy. Antibody responses to donor HLA-A, HLA-B and HLA-DRB1 antigen mismatches were common in this group of patients but the antibody reactivity was significantly lower before than after allograft removal. In contrast, we noted HLA-C reactive antibodies were less common regardless the allograft was present or had been removed. Consistent with previous observations [18], a high proportion of patients had antibodies against donor DRB3/4/5 (DR51/DR52/DR53) mismatches but the pre-allonx and post-allonx differences were small and statistically insignificant. Most patients had HLA-DQ-reactive antibodies, and their detection was slightly less in the presence of the allograft but the difference was not statistically significant.

These findings demonstrate that in patients with rejected grafts *in situ*, the incomplete detection of donor-reactive antibodies in the Luminex assay primarily apply to the HLA-A, -B and DRB1 antigens. This suggests that certain unacceptable HLA-A, -B and DR mismatches might not be defined if such patients are considered for retransplantation.

A recent paper by Billen et al. [19] describes also a markedly higher incidence of Luminex-detected DSA in post-allonx than in pre-allonx sera. This study was done on 43 cases at a single center and transplantectomy was mostly done within one year post-transplant. Only 12% of pre-allonx sera but 84% post-allonx sera had DSA against class I. For DSA against DR and DQ, these proportions were 14% and 77%, respectively. Our study yielded comparable results for post-allonx sera but the DSA frequency was much higher for pre-allonx sera. This difference might be related to cut-off criteria for assigning positive reactions in the Luminex assay. Billen et al. [19] used MFI of test bead minus MFI of negative control bead > 2000 considered positive while we assigned a positive reaction if the MFI of the test bead minus the average MFI with self-antigens was > 10% of the MFI of the positive control minus the average MFI with self-antigens. The post-transplant timing of the removal of the rejected graft might also be a factor. The cases reported by Billen et al. [19] were first transplants that had been removed within one year post-transplant. Our cases were also first transplants but they had been removed much later: 6.6 ± 5.4 years after transplantation. Only 5 of our 65 (8%) cases had allonx performed within 1 year. Therefore, our study dealt primarily with patients with a long-term transplant which as a continuous source of donor antigens could have expanded the antibody repertoire.

This type of analysis of antibody reactivity must be viewed in context with the controversy about the clinical utility of allograft nephrectomy versus leaving a rejected allograft in place. Although it is well known that nephrectomy has a significant morbidity and mortality, a recent analysis of a large database suggests that the increased risk of death applies primarily to early transplant failure patients [20]. Conversely, nephrectomy late post-transplant seems associated with more repeat transplant failures. Other reports illustrate that there is no consensus about the effect of allograft nephrectomy on retransplant survival [8,21–23]. Therefore, the clinical role of allograft nephrectomy after transplant failure remains uncertain [20].

However, it is abundantly evident that allograft nephrectomy increases the detectability of donor-specific antibodies [3–11], although more sensitive solid-phase assays such as the plate-based ELISA and the bead-based Luminex can often identify such antibodies in the presence of the transplant [17,24–32]. There are ample demonstrations that anti-donor HLA antibodies represent risk factors for rejection and graft failure, reviewed in [1], but there are also recent data that such antibodies do not affect graft survival [33] and that they can be detected in stable kidney transplant recipients[34].

Our study primarily addresses antibody detection in patients with long-term transplant failures. How do we interpret the data in relation to the determination of mismatch acceptability of a retransplant? An important issue is the clinical relevance of antibodies detected in the Luminex assay. Why did about one-half of our cases have anti-donor HLA-A, B, DR antibodies and even more had anti-DRB3/4/5 and DQ antibodies in the presence of a failed graft which should have functioned as a large sponge that absorbs such antibodies? It is possible that the detection in blood may reflect an excess of antibody not absorbed by the allograft because of low tissue expression of HLA antigens. It has been suggested that transplant tissue damaged by antibody and complement is less able to absorb antibodies from the blood [35].

Not surprisingly, the reactivity of circulating DSA was generally higher after allograft removal especially against the HLA-A, -B, and --DRB1 antigen mismatches. The most obvious explanation is that this increase involved antibodies which had been absorbed by the graft. It is tempting to speculate that such antibodies would be clinically important and that the reactive antigens should be considered as

unacceptable mismatches. Although absorbed antibodies might have caused graft failure likely through complement-dependent and other inflammatory processes, it is also possible some antibodies appeared after transplant rejection because a failed graft is a continuous source of antigen and likely the patient is on reduced immunosuppression. Another possibility is that absorbed antibodies participated in graft accommodation as a protective mechanism against transplant injury [35,36].

The Luminex assays used here have the obvious limitation that they measure only IgG-type antibodies and there is no distinction between clinically relevant Complement-binding antibodies and non-Complement-binding antibodies which have an uncertain effect on transplant outcome. Recent studies have shown that most transplant recipients develop multiple Ig types of donor-specific antibodies including IgG_{1-4} and IgA [14,37]. Classical Complement activation is a key step in the process of antibody-mediated rejection [38]. IgG1 and IgG3 and IgM type antibodies are efficient Complement binders but other factors such as antigen density on tissues and interactions between antibodies against different epitopes of the same antigen, may contribute to complement activation. A modified antigen-binding assay for C4d-fixing antibodies may identify clinically relevant antibodies [39].

This report addresses DSA in terms of reactivity with HLA antigens and we recognize the fact that HLA antibodies recognize epitopes. A detailed HLAMatchmaker-based analysis is underway to define the epitope specificity of DSA before and after allograft nephrectomy and determine the relative immunogenicity of mismatched epitopes.

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