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Higher Risk of Kidney Graft Failure in the Presence of Anti-Angiotensin II Type-1 Receptor Antibodies

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Reports have associated non-HLA antibodies, specifically those against angiotensin II type-1 receptor (AT1R), with antibody-mediated kidney graft rejection. However, association of anti-AT1R with graft failure had not been demonstrated. We tested anti-AT1R and donor-specific HLA antibodies (DSA) in pre- and posttransplant sera from 351 consecutive kidney recipients: 134 with biopsy-proven rejection and/or lesions (abnormal biopsy group [ABG]) and 217 control group (CG) patients. The ABG's rate of anti-AT1R was significantly higher than the CG's (18% vs. 6%, $p < 0.001$). Moreover, 79% of ABG patients with anti-AT1R lost their grafts (vs. 0%, CG), anti-AT1R levels in 58% of those failed grafts increasing posttransplant. With anti-AT1R detectable before DSA, time to graft failure was 31 months—but 63 months with DSA detectable before anti-AT1R. Patients with both anti-AT1R *and* DSA had lower graft survival than those with DSA alone (log-rank $p = 0.007$). Multivariate analysis showed that *de novo* anti-AT1R was an independent predictor of graft failure in the ABG, alone (HR: 6.6), and in the entire population (HR: 5.4). In conclusion, this study found significant association of anti-AT1R with graft failure. Further study is needed to establish causality between anti-AT1R and graft failure and, thus, the importance of routine anti-AT1R monitoring and therapeutic targeting.

Key words: Angiotensin II type-1 receptor antibodies, AT1R, DSA, kidney transplantation, rejection

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; Ang-II, angiotensin II; AT1R, angiotensin II type-1 receptor; DSA, donor-specific HLA antibody; MFI, mean fluorescence intensity; MTGF, mean time to graft failure; RAS, renin–angiotensin system; TCMR, T cell–mediated rejection.

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Introduction

Investigators have long stressed the importance of both non-HLA immunity and HLA immunity in transplantation (1,2). Nevertheless, compared with the abundant evidence about HLA antibodies (3–5), there is scant evidence for the impact on graft survival of humoral reactions against non-HLA targets, leaving poor understanding of failure mechanisms that HLA antibodies, alone, cannot explain. Recently, though, there has been increasing agreement that antibodies against non-HLA antigens can trigger an immunological response in solid organ transplantation (6–12).

Early studies were limited to antibodies against endothelial cells identified in heart transplantation during acute rejection, cardio allograft vasculopathy (13) and coronary heart diseases (14), and in renal transplant during hyperacute rejection (15). Recent studies have more pointedly suggested the role of antibodies in immune responses against tissue-restricted antigens (9) including vimentin (16–18), cardiac myosin (16,19,20) and the angiotensin II type-1 receptor (AT1R) (21,22). Still—as noted—the impact of these tissue-restricted antibodies on graft function and survival had not yet been demonstrated.

Because AT1R differs from all other non-HLA antigenic targets in the mechanism of humoral reactions—specifically, the binding of antibodies to AT1R induces unique physiological effects that mimic those of receptor ligand (angiotensin II [Ang-II]) in the renin–angiotensin system (RAS) (22)—we decided to explore the association of anti-AT1R with graft failure. AT1R is distributed among various organs, and is principal mediator of Ang-II's effects, causing (*inter alia*) vasoconstriction in vascular smooth muscle cells, aldosterone secretion by the adrenal cortex, and sodium reabsorption in proximal tubules (23–26). Yet disruption of the RAS leads to various pathological events including hypertension, heart failure, kidney disease, atherosclerosis, and cancer development (27–29). Induction of vascular injury by exogenous Ang-II has been thoroughly studied in animals (28,30–33). A similar role for anti-AT1R was

observed in kidney transplantation with rats that developed hypertension after passive anti-AT1R transfer (22). In transplantation, anti-AT1R was shown to be associated with antibody-mediated rejection (AMR) in the absence of DSA (21,22).

Despite these many observations, the question of how anti-AT1R development affects postrejection graft survival remained. We hypothesized that anti-AT1R, responding to graft injury caused by rejection or posttransplant lesions unrelated to rejection, may impact long-term graft outcome. This study aimed to examine the incidence of anti-AT1R and the impact of anti-AT1R on graft survival with or without histopathologic diagnoses.

Materials and Methods

Patients

Between 1999 and 2009, 471 consecutive patients received kidney transplants at East Carolina University, Brody School of Medicine/Vidant Medical Center, Greenville, NC. After excluding 120 patients on the criteria detailed in Figure 1, this study enrolled 351. These were further classified into two groups: those with abnormal biopsies (ABG, n = 134), based on biopsy-proven (BP) rejection according to Banff 97 criteria (34), or with histopathologic lesions unrelated to rejection; and a control group (CG, n = 217) comprising patients with no indication for biopsy (except for three patients whose biopsy found no abnormalities) (Figure 1). The study was approved, protocols reviewed by the Institutional Review Board. C4d staining was performed in 59 patients. Of the 351 patients, 66 suffered graft loss—defined as return to hemodialysis, nephrectomy or re-transplant (graft-loss cause in Table S1); 285 maintained functioning grafts (demographics in Table 1).

Immunosuppression

All patients received induction therapy with either rabbit anti-thymocyte globulin (ATG) or humanized anti-IL2R monoclonal antibody. Maintenance immunosuppression consisted of a calcineurin inhibitor (CsA or FK506) with a mycophenolic acid derivative. Patients received a corticosteroid taper starting at transplant. By 2 months posttransplant, the patients' prednisone level was reduced to 10 mg/day, continuing thereafter. All rejection episodes were immediately treated with corticosteroids while awaiting full biopsy results. For BP acute cellular rejection, patients received ATG with concomitant corticosteroids. For biopsies consistent with AMR, patients received plasmapheresis, ATG and costicosteroids.

HLA typing, final crossmatches and antibody screening

HLA typing (HLA-A,B,DR,DQ) of donors and recipients was accomplished by both serology and polymerase chain reaction single-antigen primer methods. The study analyzed pretransplant sera (from all except 10 patients, Figure 2) and serial sera collected posttransplant until graft loss or the end of follow-up for graft-functioning patients. Posttransplant sera included sera drawn at the time of biopsy or during biopsy diagnoses. Pretransplant PRA was determined by lymphocytotoxicity (1999–2001) or ELISA (2002–2009). T and B cell final crossmatches were performed on all recipients.

Antibody screening was performed for HLA antibodies (n = 5118, including sera collected monthly from some patients; average 14 samples/patient) using LABScreen® HLA class I and II single antigen beads (One Lambda, Inc., Canoga Park, CA) and for AT1R antibodies (n = 1792; average five samples/patient) using quantitative ELISA (CellTrend GmbH, Luckenwalde, Germany). Both procedures followed manufacturer's instructions. Briefly, anti-AT1R ELISA was performed by incubating sera (1:100) with AT1R coated on a 96-well plate for 2 h at 4°C, followed by incubation with horseradish peroxidase-conjugated anti-human IgG for 1 h at room temperature. After adding TMB substrate solution—and 20-min incubation in the dark—the plate was read with the BioTek® Microplate Reader (One Lambda, Inc.). Each ELISA was validated with one positive and one negative control (in ELISA kits). According to CellTrend GmbH, positive control was prepared by collecting positive sera comparable to the original positive control prepared from a serum sample of a patient with vascular kidney rejection (22) (1:800 dilution: 20.0 units of AT1R antibodies). Negative control was prepared from sera of healthy individuals. To

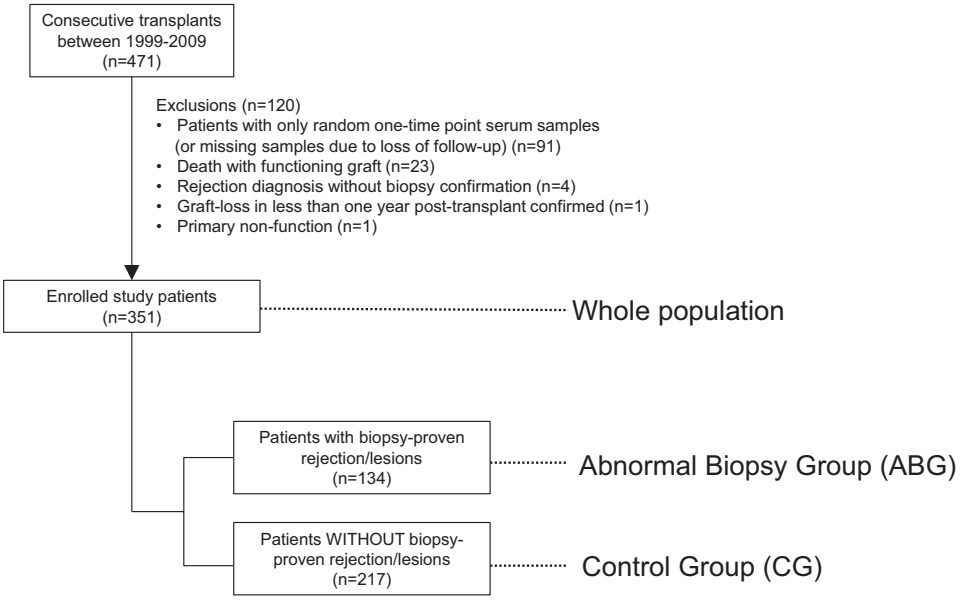

Figure 1: Flow chart of patients enrolled in the study.

Table 1: Patient baseline characteristics

	Abnormal biopsy group (ABG) (n = 134)			Control group (CG) (n = 217)		
	Anti-AT1R positive	Anti-AT1R negative	p-Value	Anti-AT1R positive	Anti-AT1R negative	p-Value
Study population						
Total number of patients	24 (100%)	110 (100%)		12(100%)	205 (100%)	
Graft-failed patients	19 (79%)	41 (37%)	<0.001	0 (0%)	6 (3%)	1.00
Patient characteristics						
Gender (male)	15 (63%)	73 (66%)	0.81	6(50%)	103 (50%)	1.00
Mean patient age (year at the time of transplant)	46.0 (±14.2)	46.2 (±13.2)	0.93	44.5 (±15.9)	50.0 (±11.0)	0.1
Patient age >45	13 (54%)	84 (76%)	0.04	7(58%)	145 (71%)	0.35
African-American	15 (63%)	74 (67%)	0.64	5(42%)	124 (60%)	0.23
Transplant characteristics						
Re-transplant	8 (33%)	11 (10%)	0.01	1(8%)	9 (4%)	0.44
Delayed graft function	1 (4%)	5 (5%)	1.00	0 (0%)	16 (8%)	0.61
CDC crossmatch	0 (0%)	0 (0%)	—	0 (0%)	0 (0%)	—
T-cell flow crossmatch[1]	0 (0%)	0 (0%)	—	0 (0%)	0 (0%)	—
B-cell flow crossmatch[1]	0 (0%)	0 (0%)	—	0 (0%)	0 (0%)	—
Pretransplant PRA >10%	5 (21%)	16 (15%)	0.53	2 (17%)	38 (19%)	1.00
Method of PRA measurement by ELISA (2002 to present)[2]	18 (75%)	82 (75%)	1.00	8 (67%)	152 (74%)	0.52
Deceased donors	11 (46%)	57 (52%)	0.66	5 (42%)	115 (56%)	0.38
Number of HLA mismatces						
HLA-A	1.1 (±0.7)	1.3 (±0.7)	0.34	1.2 (±0.7)	1.1 (±0.8)	0.72
HLA-B	1.2 (±0.8)	1.4 (±0.7)	0.20	1.4 (±0.5)	1.2 (±0.8)	0.23
HLA-DR	1.0 (±0.8)	1.1 (±0.6)	0.60	1.3 (±0.6)	1.0 (±0.7)	0.18
HLA-DQ	1.1 (±0.6)	0.9 (±0.7)	0.21	1.2 (±0.2)	0.9 (±0.6)	0.14
Immunosuppression						
Induction therapy						
Rabbit anti-thymocyte globulin	4 (17%)	16 (15%)	0.76	10 (83%)	167 (81%)	1.00
Anti-interleukin 2 receptor monoclonal antibody	20 (83%)	93 (85%)	1.00	1 (8%)	32 (16%)	0.70
Both	0 (0%)	1 (1%)	1.00	0 (0%)	6 (3%)	1.00
Maintenance regimens						
MMF, Tacrolimus (FK506)	8 (33%)	40 (36%)	0.82	4 (33%)	70 (34%)	1.00
MMF, Cyclosporin (CsA)	9 (38%)	43 (39%)	1.00	7 (58%)	110 (54%)	1.00
Others	7 (29%)	27 (25%)	0.61	1 (8%)	25 (12%)	1.00
Time factors						
Time from transplant to rejection (mean, months)	35.6 (±32.5)	19.7 (±25.7)	0.01	—	—	—
Time of follow-up after rejection (mean, months)	19.2 (±17.3)	39.2 (±31.5)	p < 0.01	—	—	—
Time of follow-up after transplantation (mean, months)	53.8 (±32.5)	58.0 (±34.0)	0.59	78.7 (±45.7)	69.1 (±37.5)	0.40
HLA antibody characteritics						
DSA positive cases (including both class I and class II)[3]	14 (58%)	47 (43%)	0.18	5 (42%)	42 (20%)	0.14
DSA-class I[4]	11	31	0.14	2	16	0.26
DSA-A	10	24	0.07	2	9	0.12
DSA-B	3	12	0.73	0	10	1.00
DSA-class II[4]	9	42	1.00	3	35	0.45
DSA-DR	2	7	0.66	1	5	0.29
DSA-DQ	8	42	0.82	2	32	1.00
Causes of ESRD						
HTN	11 (46%)	54 (49%)	0.65	8 (67%)	73 (36%)	0.03
DM	1 (4%)	6 (5%)	1.00	0 (0%)	20 (10%)	0.60
IgA nephropathy	1 (4%)	4 (4%)	1.00	0 (0%)	6 (3%)	1.00
FSGS	1 (4%)	6 (5%)	1.00	1 (8%)	9 (4%)	0.34
Others	5 (21%)	17 (15%)	0.56	2 (17%)	44 (21%)	1.00
Combination of the above causes (>two causes)	5 (21%)	20 (18%)	0.78	1 (8%)	53 (26%)	0.30

(*Continued*)

Table 1: Continued

	Abnormal biopsy group (ABG) (n = 134)			Control group (CG) (n = 217)		
	Anti-AT1R positive	Anti-AT1R negative	p-Value	Anti-AT1R positive	Anti-AT1R negative	p-Value
Rejection characteristics						
sCr (mg/dL) during biopsy diagnoses[5]	3.7 (±4.5)	3.0 (±2.7)	0.35	—	—	
sCr increase >50% baseline at time of biopsy	13 (54%)	55 (51%)	0.82	—	—	
sCr return to baseline after biopsy at month 24	4 (17%)	34 (32%)	0.21	—	—	
Noncompliance[6]	1 (4%)	8 (7%)	1.00	—	—	
Multiple rejection (>1)	7 (29%)	23 (21%)	0.42	—	—	
Histological diagnosis						
AMR[7]	2 (8%)	0 (0%)	0.03	—	—	
Probable AMR[8]	1 (4%)	0 (0%)	0.18	—	—	
DSA + CAN	1 (4%)	4 (4%)	1.00	—	—	
TCMR + negative C4d	7 (29%)	43 (39%)	0.49	—	—	
TCMR + DSA (C4d not tested)	5 (21%)	10 (9%)	0.15	—	—	
Borderline	0 (0%)	4 (4%)	1.00	—	—	
Mixed rejection	2 (8%)	12 (11%)	1.00	—	—	
Unclassified	2 (8%)	14 (13%)	0.74	—	—	
Others[9]	4 (17%)	23 (21%)	0.78	—	—	
Severity of rejection						
TCMR Type IA/IB	8 (33%)	34 (31%)	0.81	—	—	
TCMR Type IIA/IIB	5 (21%)	22 (20%)	1.00	—	—	
TCMR Type III	1 (4%)	2 (8%)	0.45	—	—	
Rejection treatment						
Rabbit anti-thymocyte globulin with corticosteroids	12 (50%)	55 (50%)	1.00	—	—	
Plasmapheresis	6 (25%)	18 (16%)	0.38	—	—	
IVIg	1 (4%)	1 (1%)	0.35	—	—	
Anti-hypertensive drugs						
Anti-hypertensives during posttransplant periods	22 (92%)	105 (95%)	0.33	—	—	
Types of anti-hypertensives during posttransplant periods						
Calcium channel blocker	20	64	0.06	—	—	
Beta blocker	17	70	0.81	—	—	
ACE inhibitor	8	23	0.28	—	—	
AT1R blocker	7	20	0.27	—	—	

Numbers are based on the total number of patients with available data. In some categories, numbers may not add up to the total due to missing information for some patients.

Abbreviations: BP, biopsy-proven; AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection; ESRD, end-stage renal disease; ACE, angiotensin-converting enzyme; AT1R, angiotensin type 1 receptor; HTN, hypertension; DM, diabetes mellitus; FSGS, focal segmental glomerulosclerosis; sCr, serum creatinine; CAN, chronic allograft nephropathy.

[1]For living donors flow crossmatches and for deceased donors lymphocytotoxicity (4 washes with anti-human globulin).

[2]PRA was determined by lymphocytotoxicity (pre-2002) or ELISA (2002 to present).

[3]All DSA are *de novo* antibodies except for 5 DSA cases (4%) that were positive both pre- and posttransplantation.

[4]Some patients developed both class I and class II antibodies.

[5]Mean of the six serum creatinine values before histopathologic diagnosis.

[6]Noncompliance with immunosuppressive therapy: There was no case in the nonrejection group.

[7]Banff classifications for AMR cases were not available.

[8]The presence of both DSA and morphologic evidence of tissue injury in the absence of C4d deposition (Banff classification).

[9]Interstitial fibrosis and tubular atrophy without immunologic activity (n = 3); Oxalosis (n = 1); FSGS (n = 3); Acute tumor necrosis (n = 5); Acute tubulointerstial nephritis (n = 2); Glomerulonephritis (n = 1); BK/polyoma nephropathy (n = 1); calcineurin-inhibitor nephrotoxicity (n = 10); arterionephrosclerosis (n = 1).

determine anti-AT1R level, the standard curve (a four-parameter logistic fit with five standard sera [at 2.5, 5, 10, 20, 40 U/mL concentration] collected from anti-AT1R-positive patients) was plotted using AT1R software, version 1.0.0 (One Lambda, Inc.). The assay was validated if the positive control ranged 15–25 U/mL and the negative control <10 U/mL. Intra-assay variability was 8.6%, inter-assay variability 7.2%. Mean fluorescence intensity (MFI) ≥1000 was considered positive for HLA antibodies, U/mL ≥15 for AT1R antibodies.

			Graft outcome	Total n (%)	Pre-/post-transplant status of anti-AT1R			Patterns of post-transplant anti-AT1R development				
					Pre-transplant positive		Pre-transplant negative					
					Pre-transplant anti-AT1R alone [1]	Pre-and post-transplant positive anti-AT1R	De novo anti-AT1R	(i) Increasing	(ii) Increasing then decreasing / fluctuating [5]	(iii) Transient [4] / fluctuating [5]	(iv) Constant [6]	(v) Decreasing
Whole population (n=351)		Total number of ***pre-transplant*** positive anti-AT1R cases (n) (Sum of [6] and [11])		60 (100%)	35 (58%)	25 (42%)	-					
		Total number of **post-*transplant*** positive anti-AT1R cases (n) (Sum of [7] and [12])		36 (100%)	-	25 (69%)	11 (31%)					
Abnormal Biopsy Group (ABG) **(n=134)**	[1]		Functioning Failed	3 0	3 0	- -	- -	- -	- -	- -	- -	- -
		Relation between anti-AT1R and DSA										
	[2]	Positive anti- AT1R alone during rejection/lesions	Functioning Failed	2 (100%) 4 (100%)	- -	2 (100%) 2 (50%)	0 (0%) 2 (50%)	0 (0%) 2 (50%)	1 (50%) 1 (25%)	1 (50%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 1 (25%)
	[3]	Both positive anti-AT1R & DSA during rejection/lesions	Functioning Failed	3 (100%) 11 (100%)	- -	2 (67%) 9 (82%)	1 (33%) 2 (18%)	0 (0%) 7 (64%)	0 (0%) 2 (18%)	3 (100%) 1 (9%)	0 (0%) 1 (9%)	0 (0%) 0 (0%)
	[4]	Positive anti-AT1R followed by DSA (n=1)[2]	Failed	1	-	1	0	0	0	0	0	1
	[5]	Positive anti-AT1R after histologic diagnoses (n=3)[3]	All failed	3	-	0	3	2	0	1	0	0
	[6]	Total number of **pre-*transplant*** positive anti-AT1R cases (sum of [1] to [4] under "Pre-transplant")		19 (100%)	3 (16%)	16 (84%)	-					
	[7]	Total number of ***post-transplant*** positive anti-AT1R cases (sum of [2] to [5])		24 (100%)	-	16 (67%)	8 (33%)					
Control Group (CG) **(n=217)**	[8]		Functioning Failed	31 1	31 1	- -	- -	- -	- -	- -	- -	- -
		Relation between anti-AT1R and DSA										
	[9]	Positive anti- AT1R alone (n=8)	Functioning Failed	8 (100%) 0 (0%)	- -	5 (63%) 0 (0%)	3 (38%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)	6 (75%) 0 (0%)	0 (0%) 0 (0%)	2 (25%) 0 (0%)
	[10]	Both positive anti-AT1R & DSA (n=4)	Functioning Failed	4 (100%) 0 (0%)	- -	4 (100%) 0 (0%)	0 (0%) 0 (0%)	1 (25%) 0 (0%)	0 (0%) 0 (0%)	1 (25%) 0 (0%)	0 (0%) 0 (0%)	2 (50%) 0 (0%)
	[11]	Total number of **pre-*transplant*** positive anti-AT1R cases (sum of [8]-[10] under "Pre-transplant")		41 (100%)	32 (78%)	9 (22%)	-					
	[12]	Total number of ***post-transplant*** positive anti-AT1R cases (n) (sum of [9] and [10])		12 (100%)	-	9 (75%)	3 (25%)					

[1]Pre-transplant serum samples were collected from 132 abnormal biopsy group patients (2 missing) and 209 control group patients (8 missing). All patients with pre-translant AT1R antibodies alone maintained good graft function except one patient in the control group.

[2]There was one patient who had positive anti-AT1R during rejection in the absence of DSA, but later during post-transplantation (22 months), DSA started to increase while anti-AT1R decreased.

[3]Three patients developed AT1R antibodies in the absence of DSA after rejection. Patient 1: 2.0 years after chronic rejection, Patient 2: 2.5 years after chronic allograft nephropathy diagnosis, and Patient 3: 1.7 years after acute rejection.

[4]Irregularly appearing peaks that became low-level before the end of follow-up or graft loss.

[5]A regular, recurring cycle of high and low levels, but overall higher levels remained similar.

[6]Higher-level AT1R antibodies (>20 U/ml) throughout post-transplantation term (no negative AT1R antibody term observed)

Figure 2: Pre - and posttransplant status of anti-AT1R and patterns of anti-AT1R development.

Statistical analysis

Continuous variables were compared with the t-test, counts and categorized variables with Fisher's exact test, survival analysis made with Kaplan–Meier estimates. Time-to-event data were compared between groups using the log-rank test. Risk factors for graft loss were assessed with Cox proportional hazard modeling. Factors found significant by univariate analysis ($p < 0.2$) received multivariate analysis with a backward stepwise Cox regression model, and were reported as a hazard ratio with a 95% confidence interval. Statistical tests used STATA/MP v10 (StataCorp, College Park, TX).

Results

Pretransplant and posttransplant AT1R antibodies

Of 351 patients, 17% (n = 60) were anti-AT1R-positive pretransplant. Of these, 58% (n = 35) were positive only pretransplant; the remaining 42% (n = 25) were anti-AT1R-positive both pre- and posttransplant (Figure 2). Just one of the patients with only pretransplant anti-AT1R had a failed graft whereas 48% (n = 12/25) with both pre- and posttransplant anti-AT1R lost their grafts. Of the 11 patients who developed *de novo* anti-AT1R (3% of the total), 64% (n = 7) lost grafts.

As noted, patients were classified into an abnormal biopsy group (ABG, n = 134) and a control group (CG, n = 217). Pretransplant anti-AT1R was positive in 14% of the ABG (19/134) and 19% of the CG (41/217) (Figure 2). In the ABG, 84% (16/19) of those positive for anti-AT1R pretransplant remained positive posttransplantation whereas only 22% (9/41) of the CG remained positive ($p < 0.001$). Except for one CG patient, all who had only pretransplant anti-AT1R maintained good graft function.

On the other hand, higher frequency of posttransplant anti-AT1R was observed in the ABG than in the CG (18% [24/134] vs. 6% [12/217]; $p < 0.001$) (Figures 2, 3A). And posttransplant anti-AT1R in 33% of those 24 ABG patients (8/24) was *de novo* (all except one graft failed), while *de novo* in only 25% (3/12) of the CG (all grafts functioning). Three ABG patients started to develop anti-AT1R more than 1 year after histologic diagnosis (Figure 2). Classification of patients by graft outcome showed that, in the ABG, 79% (19/24) with posttransplant anti-AT1R lost their grafts while none in the CG did ($p < 0.001$) (Figure 3A). Looking at overall distribution of anti-AT1R levels in the ABG, there was significant difference between patients whose grafts failed and those with functioning grafts (Mann–Whitney $p = 0.001$) (Figure 3B).

Patient and transplant characteristics

There was no significant difference in recipient and transplant characteristics between ABG and CG patients positive and negative for anti-AT1R except for older recipient age (>45) among negative patients and higher re-transplant rate among positive ABG patients (Table 1). Comparison of anti-AT1R frequency between primary and re-transplant patients showed a significantly higher rate of anti-AT1R in re-transplant patients than in primary transplant patients (31% [9/29] vs. 8% [27/322], $p = 0.001$). Prehypertension most commonly caused end-stage renal disease among CG patients positive for anti-AT1R. In the ABG, T cell–mediated rejection (TCMR) was the most common histological diagnosis in both anti-AT1R-positive and -negative patients, but AMR and probable AMR (defined in Table 1; n = 3) were observed only in positive patients.

Patterns of anti-AT1R development

In the ABG, patterns of increasing anti-AT1R development were observed only among graft-failed patients (46%, 11/24), whereas one functioning-graft CG patient had an increasing pattern (Figure 2).

Overall, ABG anti-AT1R-positive patients had a significant higher rate of increasing anti-AT1R pattern than CG patients (46% vs. 8%, $p = 0.03$).

Of 11 graft-failed patients with both anti-AT1R and DSA (Figure 2), seven (64%) had increasing levels of anti-AT1R with subsequent graft loss, while the remaining four (36%) developed anti-ATR in patterns that were increasing then decreasing (n = 2), transient/fluctuating (n = 1) or constant (n = 1) (Figure 2). Furthermore, of the seven graft-failed patients who developed increasing anti-AT1R and DSA, three had both increasing anti-AT1R and DSA, whereas anti-AT1R increased in the remaining four while DSA decreased (Figure 4).

Anti-AT1R in the absence of DSA

Six patients had anti-AT1R with no DSA during BP-rejection/lesions, with four graft failures (Figure 2). Except in one patient (graft failed in 5 months), anti-AT1R level increased before a >25% elevation of serum creatinine (sCr) that continued to rise before graft failure. The first patient experienced mixed rejection with sCr increase from 2.5 mg/dL baseline (last 6 years) to 24.1 mg/dL 1 month after an increase of anti-AT1R level (from 1.5 U/mL, 3 months posttransplant, to 8.3 U/mL 90 months posttransplant). In 2.5 months, the AT1R level reached 19 U/mL with hypertension progressing to stage 2 and subsequent failure. Biopsy of the second patient found focal segmental glomerulosclerosis and calcineurin-inhibitor nephrotoxicity (CNIT) after 7 months of increasing anti-AT1R (from 12 U/mL, 2.4 months posttransplant, to 40 U/mL 39 months posttransplant); in just 3 months, sCr increased from 1.5 to 7.6 mg/dL, followed by graft failure. The third patient's baseline sCr started to increase from 1.1 mg/dL (last 6 years) to 1.7 mg/dL with a diagnosis of vascular type IIA 10 months after anti-AT1R (26.1 U/mL) was first detectable; then the graft failed. Thus, in all three cases, anti-AT1R development preceded sCr increase with subsequent graft failure.

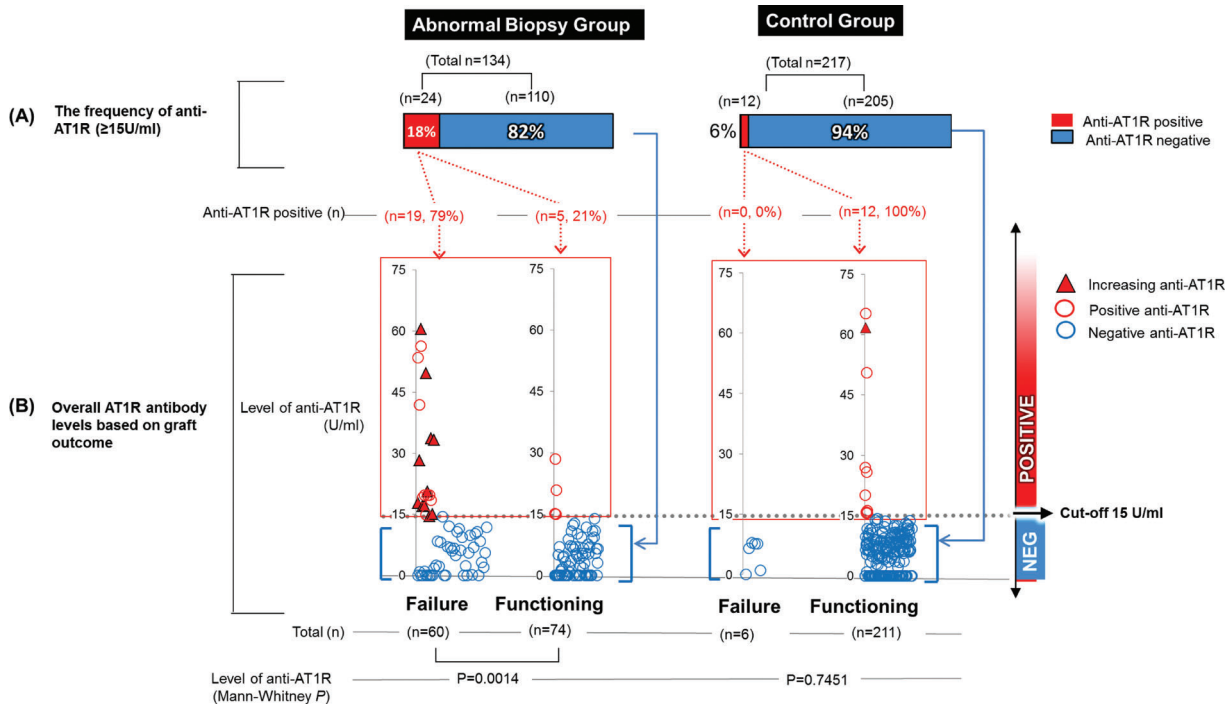

Figure 3: Comparison of AT1R antibody levels between patients with graft failure and with functioning grafts. (A) In the abnormal biopsy group (ABG), of patients who experienced biopsy-proven (BP) rejection/lesions, 18% (24/134) were positive for AT1R antibodies. Of those 24 patients, 19 (79%) lost grafts and only 5 (21%) maintained good graft function. In the control group (CG), of patients who did not have histopathologic abnormalities, only 6% (12/217) were positive for AT1R antibodies, and all maintained good graft function. (B) Among the ABG, significantly higher levels of AT1R antibodies were observed in the graft-failed group than in the graft-functioning group (Mann–Whitney $p = 0.0014$).

Table 2: Comparison of the time points when four posttransplant events were first detectable—development of anti-AT1R and of DSA, histologic diagnoses and graft failure

The order of antibody appearance	Post-tx or pre-tx	n	Rejection	Months to the events from transplantation: Anti-AT1R	DSA	Rejection	GF	MTGF (months)	
Anti-AT1R → DSA	Post-tx anti-AT1R → Post-tx DSA	4	TCMR (IIA)	2.3	9.3	17.1	36.9	36.5	31.0
			TCMR (IA)	3.6	21.7	5.5	24.4		
			CNIT	4.1	19.5	19.5	60.0		
			CNIT	14.1	21.6	9.8	24.7		
	Pre-tx anti-AT1R* → Post-tx DSA	4	TCMR (IA)	0.0	16.9	17.3	31.9	25.5	
			TCMR (IA)	0.0	7.9	7.1	30.0		
			TCMR (IA)	0.0	20.1	18.8	25.8		
			TCMR (IB)	0.0	4.0	3.6	14.1		
DSA → anti-AT1R	Post-tx DSA → Post-tx anti-AT1R	2	AMR	43.7	31.4	40.3	43.7	77.6	62.5
			TCMR (IA)	3.0	0.8	106.4	111.4		
	Pre-tx DSA → Pre-tx anti-AT1R*	2	TCMR (IIA)	0.0	0.0	51.1	51.7	47.5	
			Probable AMR, CNIT	0.0	0.0	35.1	43.2		

Abbreviations: GF, graft failure; AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection; CNIT, calcineurin-inhibitor nephrotoxicity; MTGF, mean time to graft failure.
*Pretransplant anti-AT1R continued to be positive during posttransplantation term (i.e. positive anti-AT1R both pre- and posttransplant).

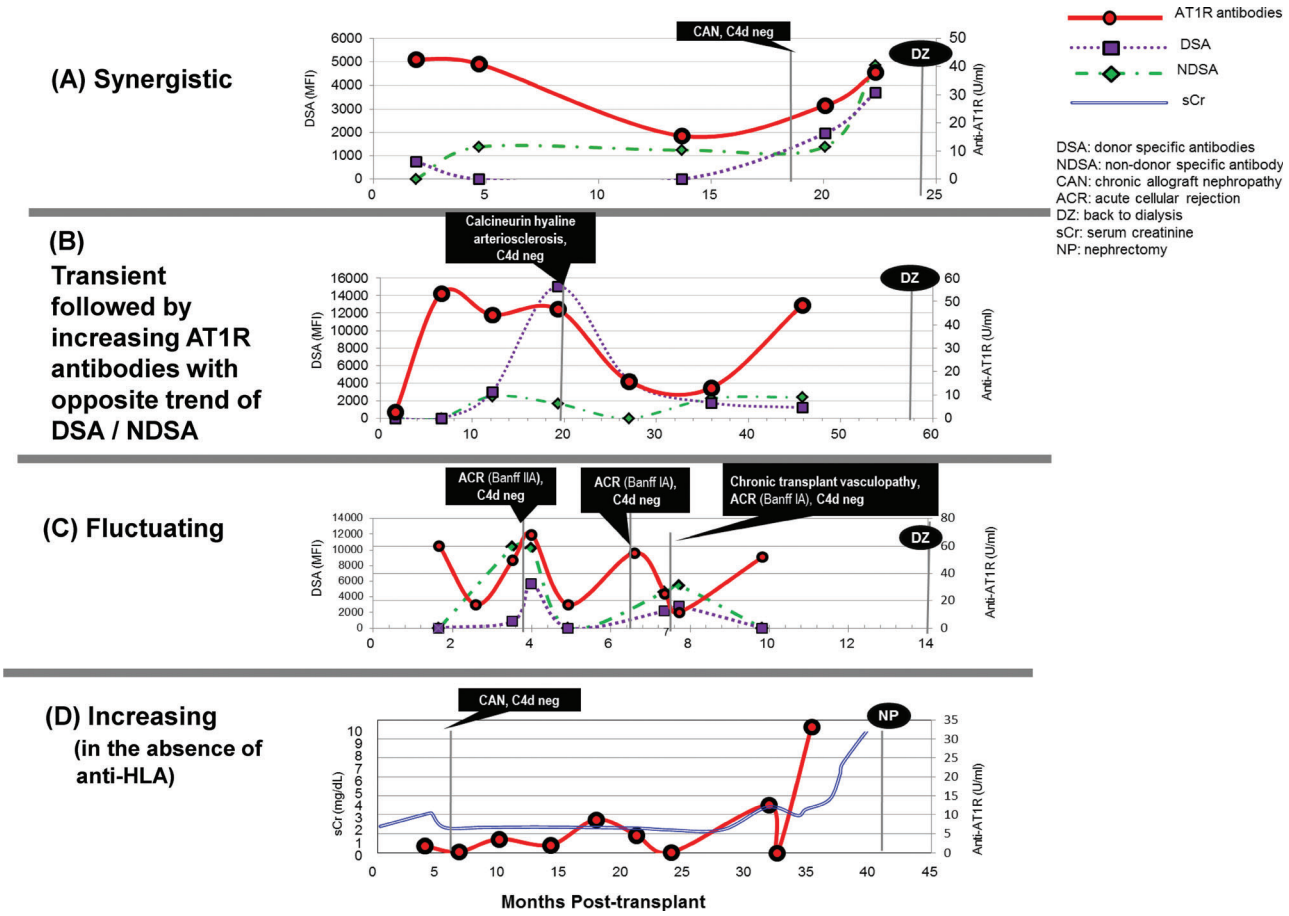

Figure 4: Patterns of AT1R antibody development. (A) Tandem development of AT1R antibodies and DSA was observed before graft failure. At 14 months posttransplant, when the AT1R antibody level was 15 U/mL, DSA (DQ4) was completely negative whereas NDSA (B82) was positive (MFI 1240). At 18.3 months posttransplant, the patient was diagnosed with chronic allograft nephropathy in the absence of C4d staining. The strength of all—anti-AT1R and both class I and class II DSA—increased as graft failure neared. (B) Transient followed by increasing AT1R antibodies and the opposite trend with AT1R antibody from DSA and NDSA. The patient had detectable pretransplant AT1R antibody, but it remained completely negative for 7 months posttransplant. In 7 months, the levels of AT1R antibodies jumped to 54 U/mL (serum creatinine [sCr] level 2.1 mg/dL), and 27 days later, biopsy diagnosis found calcineurin hyaline arteriosclerosis and interstitial fibrosis/tubular atrophy. SCr level started to increase, reaching 3.4 mg/dL. The high levels of AT1R antibodies (>40 U/mL) dropped to less than 16 U/mL after 8 months but started to increase again to 48 U/mL in 10 months. Only AT1R antibodies remained at a higher level before graft failure while DSA were decreasing. (C) Fluctuating development of anti-AT1R in the presence of DSA and the occurrence of multiple rejections with negative C4d. The patients experienced a total of three ACRs in the absence of C4d staining. AT1R antibody levels became very high during the first two ACRs (68.4 U/mL in 4 months posttransplantation, and 55.3 U/mL in 7 months posttransplant). (D) There was no significant sCr baseline change after the first diagnosis of chronic allograft nephropathy (5 months posttransplant) with low-level AT1R antibodies in the absence of HLA antibodies. However, after several small fluctuations, AT1R antibody level dramatically increased from an undetectable level to high-level 33 U/mL in just 3 months (35 months posttransplantation). Six months later, the highest sCr baseline change was observed followed by nephrectomy.

Time points when four transplant events were first detected: appearance of anti-AT1R and of DSA, occurrence of rejection/lesions and diagnosis of graft failure

Eleven ABG patients developed both DSA and anti-AT1R before graft failure (Figure 2); one developed only anti-AT1R during rejection but 18 months later developed DSA. Eight (67%, 8/12) were diagnosed with TCMR; four (33%, 4/12) experienced AMR, probable AMR or CNIT. When newly developed anti-AT1R antibodies were classified as either *de novo* or ''rebound'' anti-AT1R (i.e. patients positive for anti-AT1R pretransplant, but negative for <12 months after transplantation, then positive again), in six of those with both anti-AT1R and DSA, first detectable anti-AT1R appeared posttransplant (Table 2). In the remaining six, anti-AT1R was detected pretransplant and continued

positive posttransplant. Thus, anti-AT1R developed before DSA in eight patients, whereas DSA developed before anti-AT1R or together with anti-AT1R pretransplant in four patients (Table 2).

The two patients with posttransplant DSA detectable before posttransplant anti-AT1R were diagnosed with graft failure much later than the four with detectable posttransplant anti-AT1R before posttransplant DSA: mean time to graft failure (MTGF), respectively, 77.6 months and 36.5 months. Of the six patients positive for anti-AT1R both pre- and posttransplant, the two with both DSA and anti-AT1R pretransplant experienced rejection—either TCMR or AMR—later (MTGF: 47.5 months), meaning longer graft survival for them than for those who experienced TCMR and developed DSA posttransplant after pretransplant anti-AT1R (MTGF: 25.5 months) (Table 2). Thus, patients with anti-AT1R detected before DSA had graft failure much sooner (31 months) than those with detectable DSA before anti-AT1R or pretransplant DSA (63 months).

Impact of anti-AT1R on graft survival with DSA present or absent

Kaplan–Meier graft-survival analysis showed better survival for ABG patients with neither DSA nor posttransplant anti-AT1R (Figure 5). So, unsurprisingly, the lowest graft survival was observed among patients who developed both DSA and posttransplant anti-AT1R (log-rank $p < 0.001$). Especially, survival with both antibodies was significantly lower than survival with DSA alone (log-rank $p = 0.007$, Figure 5).

Univariate analysis further classified anti-AT1R as *de novo*, pre- and posttransplant, or positive only pretransplant. First, univariate analysis of the ABG ($n = 134$) showed significant association with graft failure that was patient-related: age, gender, ethnicity and primary disease; transplant-related: deceased donor, re-transplant, delayed graft function (DGF) and number of HLA-DQ mismatches; antibody-related: DSA, *de novo* anti-AT1R and pre- and posttransplant anti-AT1R and, finally, rejection-related: multiple rejections and chronic injuries ($p < 0.2$). Multivariate analysis showed that *de novo* anti-AT1R, pre- and posttransplant anti-AT1R, DSA, DGF and deceased donor were independent predictors, with development of *de novo* anti-AT1R posing the greatest risk of graft loss (HR = 6.62, $p = 0.001$) (Table 3).

Univariate analysis of the whole population ($n = 351$) showed many more factors associated with graft failure. These were patient-related: ethnicity and primary disease; transplant-related: deceased donor, re-transplant and number of both HLA class I and class II mismatches; immunosuppression; antibody-related: DSA, *de novo* anti-AT1R, pre- and posttransplant anti-AT1R and positive AT1R antibodies only pretransplant and, finally, rejection-related: multiple rejections, chronic injuries and severity of rejection ($p < 0.2$). Of those, five factors were independently associated with graft failure: *de novo* anti-AT1R, DSA, African-American ethnicity, multiple rejection and chronic injuries, with development of *de novo* anti-AT1R remaining the highest risk of graft loss (HR = 5.35, $p < 0.0001$) (Table 3). Having anti-AT1R only pretransplant and MMF + CsA favorably affected graft survival.

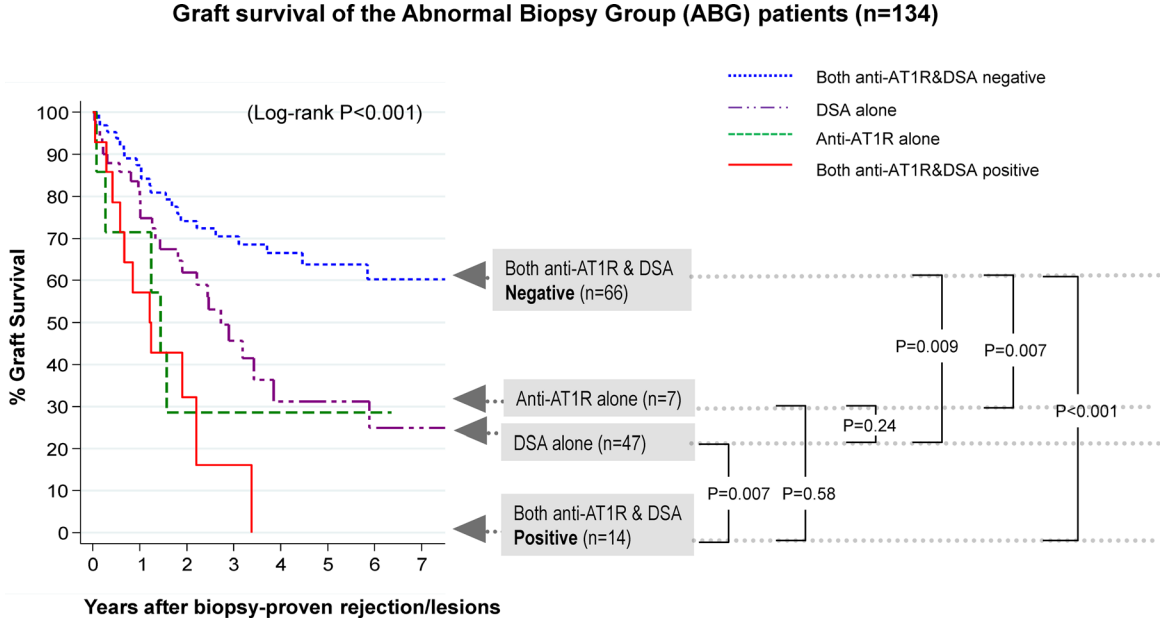

Figure 5: Kaplan–Meier graft survival based on DSA and anti-AT1R. In the ABG, patients with neither DSA nor AT1R antibodies had the best graft survival. In contrast, those who developed both DSA and anti-AT1R had the lowest survival rate (log-rank $p < 0.001$). The survival in the presence of both DSA and AT1R antibodies was significantly lower than among patients with DSA alone ($p = 0.007$).

Table 3: Factors associated with graft failure based on Cox proportional hazard analysis

	Abnormal biopsy group (n = 134)						Whole population (n = 351)					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
Variable	HR	[95% CI]	P	HR	[95% CI]	P	HR	[95% CI]	P	HR	[95% CI]	P
Patient-related												
Recipient age at the time of transplant (>45)	0.67	0.39–1.16	0.153[a]	—	—	—	1.05	0.62–1.78	0.859			
Male gender	0.71	0.42–1.20	0.199[a]	—	—	—	1.37	0.83–2.25	0.217			
African-American race	2.23	1.20–4.13	0.011[a]	—	—	—	2.28	1.28–4.06	0.005[a]	2.75	1.47–5.16	0.002
Primary disease												
Hypertension	0.96	0.57–1.63	0.891				1.62	0.99–2.66	0.057[a]	—	—	—
Diabetes mellitus	0.79	0.19–3.27	0.748				0.47	0.12–1.95	0.300			
IgA nephropathy	0.92	0.22–3.80	0.911				1.09	0.27–4.46	0.906			
FSGS	0.45	0.11–1.86	0.271				0.41	0.10–1.68	0.213			
Others	0.67	0.30–1.48	0.324				0.52	0.24–1.15	0.108[a]	—	—	—
Multiple ESRD (combinations of the above)	1.69	0.94–3.07	0.082[a]	—	—	—	1.07	0.61–1.89	0.81			
Transplant-related												
Deceased donor	1.91	1.13–3.23	0.016[a]	1.97	1.11–3.50	0.020	1.46	0.89–2.37	0.133[a]	—	—	—
Re-transplant	2.29	1.26–4.18	0.007[a]	—	—	—	3.95	2.21–7.05	<0.0001[a]	—	—	—
Pretransplant PRA >10%	1.31	0.66–2.59	0.438				1.10	0.59–2.06	0.758			
Delayed graft function (DGF)	2.65	1.05–6.64	0.038[a]	3.85	1.48–10.05	0.006	1.14	0.46–2.85	0.774			
Number of HLA mismatches												
HLA-A	1.23	0.85–1.77	0.272				1.62	1.15–2.29	0.006[a]	—	—	—
HLA-B	1.13	0.77–1.67	0.532				1.50	1.05–2.14	0.025[a]	—	—	—
HLA-DR	1.05	0.71–1.54	0.818				1.32	0.92–1.89	0.128[a]	—	—	—
HLA-DQ	1.45	0.99–2.14	0.057[a]	—	—	—	1.56	1.09–2.25	0.016[a]	—	—	—
Immunosuppressiom												
MMF, Cyclosporin (CsA)	0.71	0.41–1.24	0.230				0.52	0.32–0.87	0.013[a]	0.52	0.29–0.91	0.023
MMF, Tacrolimus (FK506)	1.05	0.61–1.81	0.850				1.07	0.63–1.79	0.809			
Others	1.34	0.79–2.29	0.279				2.11	1.26–3.52	0.004[a]	—	—	—
Induction therapy							0.85	0.48–1.52	0.589			
Rabbit anti-thymocyte globulin	1.07	0.56–2.07	0.830				1.16	0.62–2.17	0.641			
Anti-interleukin 2 receptor monoclonal antibody	0.91	0.48–1.72	0.775				0.90	0.49–1.66	0.744			
Both	1.31	0.18–9.51	0.790				0.72	0.10–5.22	0.748			
Antibody-related												
DSA[b]	2.33	1.38–3.95	0.002[a]	2.01	1.14–3.55	0.016	5.73	3.44–9.56	<0.0001[a]	3.89	2.24–6.77	<0.0001
de novo AT1R antibodies	4.02	1.42–11.39	0.009[a]	6.62	2.10–20.87	0.001	6.73	3.03–14.93	<0.0001[a]	5.35	2.16–13.25	<0.0001
Pre- and posttransplant positive AT1R antibodies	2.82	1.48–5.37	0.002[a]	2.98	1.51–5.88	0.002	2.80	1.49–5.23	0.001[a]	—	—	—
Positive AT1R antibodies only pretransplant[c]	—	—	—				0.09	0.01–0.64	0.017[a]	0.10	0.01–0.72	0.023
Rejection-related												
Multiple rejection (>2)	1.52	0.88–2.63	0.132[a]	—	—	—	6.99	4.06–12.02	<0.0001[a]	2.32	1.20–4.49	0.012
Chronic injuries[d]	1.78	1.07–2.98	0.027[a]	—	—	—	6.65	4.09–10.80	<0.0001[a]	3.34	1.85–6.02	<0.0001
Rejection severity (Banff score)	1.01	0.76–1.34	0.958									
TCMR Type IA/IB	0.94	0.54–1.66	0.844				3.35	1.92–5.86	<0.0001[a]	—	—	—
TCMR Type IIA/IIB or III	0.99	0.54–1.80	0.961				3.94	2.20–7.05	<0.0001[a]	—	—	—

Abnormal biopsy group (n = 134): patients with biopsy-proven rejection/lesions only.
Whole population (n = 351): patients with or without biopsy-proven rejection/lesions (abnormal biopsy group and control group combined).
Abbreviations: HR, hazard ratio; CI, confidence interval; ESRD, end-stage of renal disease; TCMR, T cell–mediated rejection; FSGS, focal segmental glomerulosclerosis; IF/TA, interstitial fibrosis and tubular atrophy without immunologic activity; CNI, calcineurin inhibitor.
[a]p-Values <0.2 were subjected to a backward stepwise Cox regression model (multivariate analysis).
[b]DSA cases include both class I DSA and class II DSA.
[c]In the abnormal biopsy group (ABG), there are only three patients who had pre-AT1R antibodies alone (disappeared after transplantation).
[d]Transplant glomerulopathy or vasculopathy (n = 5); BK/polyoma nephropathy (n = 5); calcineurin-inhibitor-induced nephrotoxicity (n = 10); chronic allograft nephropathy or unclassified chronic rejection (n = 36).

Discussion

This study's longitudinal analysis of anti-AT1R explored the long-term effect of non-HLA anti-AT1R antibodies, not just their role during rejection as other studies have done. To examine the effect of graft injury on anti-AT1R development—evidenced by histological abnormalities—analysis compared the ABG and CG. Then the impact of

posttransplant anti-AT1R on graft survival was examined in the whole population. While many longitudinal studies have examined the effect of HLA antibodies (35–37), this is first to show that non-HLA anti-AT1R antibodies, also, are significantly associated with graft failure. However, it should be emphasized that this study found only anti-AT1R's association with, not causality of, graft failure. Causality needs to be further investigated to assess the clinical importance of monitoring AT1R antibodies for better predictions of graft outcomes.

Association of anti-AT1R and graft failure

Previous reports on anti-AT1R in pretransplant sera and during rejection showed significant association of high-level anti-AT1R with AMR even in the absence of DSA (21,22). Our study went further, examining posttransplant serial sera as well as pretransplant sera, finding that a majority of patients with anti-AT1R experienced graft failure *after* BP rejection or lesions. First, a much higher rate (79%) of both TCMR and AMR patients who developed anti-AT1R during rejection or from lesions lost grafts. Second, ABG patients who developed both DSA and anti-AT1R had the worst survival, suggesting the synergistic detrimental effect of HLA and non-HLA antibodies to produce poor graft survival. Third, multivariate analysis confirmed that both anti-AT1R and DSA are independent predictors of poor graft survival; *de novo* anti-AT1R represented the highest risk of graft failure in both the ABG and the whole population.

Patterns of anti-AT1R development

All patterns of increasing posttransplant anti-AT1R were observed only in graft-failed patients except for one functioning-graft patient. Patterns of developing anti-AT1R in the other graft-functioning patients were transient, fluctuating or decreasing. Moreover, a significantly higher rate of patterns of increasing anti-AT1R development was observed in the ABG. Increasing anti-AT1R levels in graft failure may be comparable to the pattern observed in graft-failed patients with increasing HLA antibodies, as shown in the graft-failed patients who had distinctively increasing DSA (38) and significantly increasing HLA and MICA antibodies with graft failure (36). It is plausible that graft rejection or injuries caused by creation of newly exposed antigenic sites may have triggered new anti-AT1R production.

Pretransplant anti-AT1R

Our study showed that 75% of the graft-failed patients with detectable anti-AT1R during BP-rejection/lesions were also positive for pretransplant anti-AT1R (Figure 2). The higher rate of pretransplant anti-AT1R was not surprising since previous studies have reported high levels of pretransplant anti-AT1R in 33% of kidney recipients (21) and 81% of patients with malignant hypertension and refractory vascular rejection absent HLA antibodies (22). Moreover, the latter study showed agonistic response of IgG both pretransplant and during rejection (22). In cardiac transplantation, patients with any grade of acute rejection had significantly higher levels of AT1R and endothelin-1 type A receptor antibodies during the first year posttransplant than patients without acute cellular rejection (39). The significant difference between our ABG and CG is that, despite the similar rate of pretransplant anti-AT1R, a majority (84%) of ABG patients with pretransplant anti-AT1R remained positive posttransplant, whereas only 22% CG pretransplant-positive patients remained positive posttransplant. All these observations, together—including shorter MTGF in those developing pretransplant anti-AT1R before posttransplant DSA—may indicate that pretransplant anti-AT1R can be agonistic before transplantation or can become agonistic after transplantation, although *de novo* anti-AT1R proved the highest risk for graft loss.

Cause-and-effect relationship between DSA, anti-AT1R, and injuries

Nath et al. (16) suggested a causal relationship between HLA allo-antibodies and non-HLA auto-antibodies, showing that damage caused by HLA antibodies results in abnormal exposure of self-proteins, eliciting an autoimmune response (7)—demonstrated by observation of HLA antibodies that preceded K-α-1-tubulin auto-antibodies and by a separate observation of the role of vimentin and myosin antibodies in cardiac allograft recipients.

In contradistinction, we found that HLA antibodies did not always precede antibodies against non-HLA antigens: 67% of patients with anti-AT1R and DSA posttransplant had anti-AT1R detected before DSA, and their MTGF was much shorter than that of those who developed DSA first. Earlier graft loss was also observed in those who had pretransplant anti-AT1R and posttransplant DSA than in those who had both anti-AT1R and DSA pretransplant. Together, these findings suggest that anti-AT1R is not always the secondary effect of tissue injuries caused by anti-HLA or other factors, but that, in other mechanisms, anti-AT1R acts as primary cause of graft injury in response to stimuli related to primary diseases, polymorphisms in RAS (40) or transplants. Note that significantly higher rates of anti-AT1R were observed in re-transplant recipients compared with primary transplant recipients (31% vs. 8%, $p = 0.001$). Furthermore, rates of re-transplant were higher in the ABG patients, especially in anti-AT1R-positive patients with higher rates of graft failure (7/8). Together, the data suggested that tissue injury resulting from previous graft failure/rejection might be a cause of anti-AT1R production that promotes secondary tissue damage.

The difference in observations between previous reports (7,16) and ours may indicate a different pathogenesis of organ damage in auto-antibody activation between hearts and kidneys. Alternatively, different types of antigenic targets may promote different patterns of antibody production. In any case, further study of anti-

AT1R is needed to elucidate whether anti-AT1R antibodies cause further graft damage or it is the consequence of other mechanisms—or both.

Functioning in the presence of anti-AT1R

We also found some patients with good graft function despite the presence of anti-AT1R. A similar phenomenon was observed with the sera of healthy individuals: A few had high-level anti-AT1R despite their healthy condition (data not shown). All CG patients positive for anti-AT1R (6% of the total) retained functioning grafts, whereas 79% of ABG patients positive for anti-AT1R had failed grafts. This certainly raises the question of why some patients maintained stable grafts despite the presence of anti-AT1R. Perhaps positive anti-AT1R antibodies, as defined by their levels, were not all equal: the levels of positive anti-AT1R appeared quantitatively similar, but functional properties of anti-AT1R in both groups may be different as seen in the different patterns of anti-AT1R development (i.e. increasing concentration) during posttransplantation as well as the higher incidence of concurrent anti-AT1R and DSA in the ABG. AT1R-IgG subclasses may affect the overall function of AT1R antibodies to stratify anti-AT1R into activating or nonactivating antibodies (22). We identified a potential protecting role of a particular AT1R-IgG subclass (data not shown).

It remains unclear whether other factors affect the characteristics of anti-AT1R, making them more agonistic than others. First, the affinity of antibodies may be affected by the level of AT1R activation. The tissue damage caused by certain mechanisms prior to anti-AT1R binding may affect the level of AT1R expression, resulting in different degrees of anti-AT1R binding efficiency. Elevated expression of AT1R was observed in rejection of heart transplants (41); untreated obese rat kidney had increased AT1R mRNA expression (42). In our study, mixed patterns of the first appearance of anti-AT1R may result from altered AT1R expression caused by preexisting diseases, HLA-DSA or rejection. It is also important to find out how downstream transcription factors in RAS activation (43,44) including ERK 1/2 phosphorylation, up- or down-regulate the effects caused by anti-AT1R in transplantation (45). All these findings suggest the need to investigate how the presence of anti-AT1R, the expression of AT1R, and the induction of regulators act together to promote graft injury.

Limitations

This study found an association of anti-AT1R with poor graft survival, but causality between anti-AT1R and graft failure—which would justify routine monitoring of this antibody and identification of efficient therapeutic targets—remains unproven. Further limitations include: (1) no accurate assessment of C4d deposition in anti-AT1R-positive AMR; (2) unknown monthly shift of anti-AT1R, blood pressure levels and proteinuria due to annual serum collection; (3) unknown DSA against HLA-C, -DP and -DQA; (4) incomplete phenotype information in biopsy diagnoses, especially chronic injuries; (5) no simultaneous monitoring of antibodies and histologic abnormalities.

Conclusion

Our study showed a significant association of anti-AT1R, specifically *de novo* anti-AT1R and graft failure as a potential risk factor after rejection and/or lesions. Lowest graft survival among patients who developed both anti-AT1R and DSA suggests the synergistic detrimental effect of HLA and non-HLA anti-AT1R on graft survival. However, the justification for routine monitoring of anti-AT1R awaits definitive proof of causality of graft failure by anti-AT1R.

Disclosure

Although the authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*, at the time it was first written, M. Taniguchi was employed by One Lambda, Inc., manufacturer of assay material used in the study, and P.I. Terasaki was chairman and principal stockholder of One Lambda. Those connections ceased with the sale of One Lambda to Thermo Fisher Scientific, Inc., in September 2012. Neither M.T. nor P.I.T. is connected with Thermo Fisher.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1: Cause of graft failure