Non-HLA Agonistic Anti-Angiotensin II Type 1 Receptor Antibodies Induce a Distinctive Phenotype of Antibody-mediated Rejection in Kidney Transplant Recipients

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Anti-angiotensin II type 1 receptor (AT1R) antibodies have been associated with allograft rejection. We hypothesized that circulating AT1R antibodies might identify kidney transplant recipients at increased risk of allograft rejection and loss who are not identified by the HLA system. We prospectively enrolled 1845 kidney transplant recipients from two centers. Donor-specific HLA antibodies (DSAs) and AT1R antibodies were measured at the time of the first acute rejection episode or at 1 year post-transplant. Allograft biopsy was performed to evaluate the rejection phenotype and to assess for endothelial activation. Overall, 371 (20.1%) participants had AT1R antibodies, 334 (18.1%) had DSAs, and 133 (7.2%) had both. AT1R antibodies were associated with an increased risk of allograft loss (adjusted HR 1.49, 95% CI 1.07-2.06 for AT1R antibodies alone and 2.26, 95% CI 1.52-3.36 for AT1R antibodies and DSAs). Participants with AT1R antibodies had a higher incidence of antibody-mediated rejection (AMR) compared with participants without AT1R antibodies (25.0% vs. 12.9%). Among 77 participants with histological features of AMR but without DSAs, 51 (66.2%) had AT1R antibodies. Compared to participants with prototypical DSA-mediated rejection, those with AT1R antibody-associated rejection had a higher prevalence of hypertension, more vascular rejection with arterial inflammation, higher levels of endothelial-associated transcripts, and lack of complement deposition in allograft capillaries. Thus, AT1R antibodies may identify kidney transplant recipients at high risk of allograft rejection and loss, independent of the HLA system. Recognition of complement-independent AT1R antibody-mediated vascular rejection could lead to the development of new treatment strategies to improve allograft survival.

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Incompatibility between genetically disparate donor and recipient pairs has been recognized as the fundamental contributing factor to allograft rejection, which is a main impediment to successful transplantation of various organs, including kidney,[1] heart,[2] lung,[3] and liver.[4] Allograft rejection mainly occurs through the triggering of donor-specific immune responses mediated by T and B cells.[5] The human leukocyte antigen (HLA) tissue system has been identified as the primary target of donor-specific alloimmune responses, especially by involving recipient production of antibodies directed toward non-self-donor HLA class I and class II proteins that are expressed on the allograft endothelium and show high polymorphism.[6] The assessment of the HLA system, which consists of measuring the degree of HLA class I and II tissue antigen matching between the recipient and the donor and then detecting circulating donor-specific anti-HLA antibodies (DSAs) in the recipient's serum, represents the contemporary gold standard for defining patients' access to transplantation, post-transplant immunologic monitoring, and allograft rejection diagnosis.[6,7] However, it is commonly acknowledged that this HLA-centric approach does not provide a sufficient level of accuracy for the evaluation of immunologic complications in transplant recipients.[7] Accumulating evidence has highlighted that allograft rejection with

pathologic features of antibody-mediated rejection can occur in patients without detectable DSAs using highly sensitive contemporary assays,[8] even in recipients of transplants from HLA-identical siblings.[9]

The allograft endothelium represents the first barrier between the transplanted organ and the immune system of the recipient; this endothelium expresses the most potentially relevant antigens in transplant immunobiology.[5] Among antibodies to non-HLA endothelial antigens, agonistic antibodies directed toward angiotensin II type 1 receptor (AT1R), a G-protein coupled receptor that is expressed at the endothelial cell surface, have been the most commonly reported non-HLA antibodies associated with allograft rejection. The presence of anti-AT1R antibodies in transplant recipients was initially reported in 16 patients with steroid-refractory vascular rejection[10] and was further associated in small studies with the occurrence of allograft dysfunction and rejection or loss in adult and pediatric kidney,[11–23] heart,[24] lung,[25] and liver[26] transplant recipients. However, these findings have not been reproduced by other investigators, who reported a lack of association between anti-AT1R antibodies and allograft outcomes or associations limited to patients with concomitant DSAs,[27–30] particularly in the largest study conducted to date.[31] Therefore, the impact of anti-AT1R antibodies on transplant outcomes at the population level, with respect to the presence of concomitant anti-HLA antibodies and to what extent they can induce allograft rejection independently and/or synergistically with anti-HLA antibodies, remains largely unclear. Given the low level of evidence linking anti-AT1R antibodies to allograft rejection and loss at the population level, screening for these antibodies is neither considered standard of care for risk stratification and monitoring of allograft rejection by international guidelines[7,32,33] nor for the diagnosis of allograft rejection by international classifications.[34–37] We sought to determine, in a large, prospective cohort of well-phenotyped kidney transplant recipients, whether post-transplant circulating anti-AT1R antibodies might play a role in the failure of kidney allografts and to what extent their detection might identify patients at risk of allograft rejection and loss who are unrecognized by current diagnostic standards based on HLA assessment.

RESULTS

Patient characteristics

Among 1845 kidney recipients transplanted between January 1, 2008 and December 31, 2012, we identified 504 patients (27.3%) with post-transplant anti-AT1R antibodies and 1341 patients (72.7%) without post-transplant anti-AT1R antibodies. Table 1 shows the demographic and clinical characteristics of the patients at the time of allograft evaluation according to the presence of post-transplant anti-AT1R antibodies. Compared with patients without post-transplant anti-AT1R antibodies, patients with post-transplant anti-AT1R antibodies were significantly younger (49.3 [12.6] vs. 46.9 [14.5] years old, respectively; $P = 0.001$), more often women (497/1341 [37.1%] vs. 247/504 [49.0%]; $P < 0.001$), had a higher prevalence of previous kidney transplantation (216/1341 [16.1%] vs. 118/504 [23.4%]; $P < 0.001$), and showed a higher level of sensitization against HLA antigens at the time of transplantation, with a mean calculated panel of reactive HLA antibodies of 38.3% (34.2%) versus 45.2% (36.3%; $P < 0.001$).

Kidney allograft survival

The median follow-up after antibody screening was 7.23 years (interquartile range, 4.13–9.03). Figure 1a shows kidney allograft survival according to anti-AT1R antibody status after transplantation. Patients with post-transplant anti-AT1R antibodies had decreased kidney allograft survival compared with patients without post-transplant anti-AT1R antibodies (7-year allograft survival after antibody screening of 76.4% [95% confidence interval {CI}, 72.1–80.1] vs. 84.8% [95% CI, 82.6–86.7], $P < 0.001$). Higher levels of circulating anti-AT1R antibodies were associated with increasing incidence of allograft loss (Supplementary Figure S1). Patients with anti-AT1R antibodies and DSAs had the worst allograft survival after antibody screening (60.2%; 95% CI, 50.4–68.7) compared with patients with only DSAs (75.3%; 95% CI, 69.9–79.8), those with only anti-AT1R antibodies (81.9%, 95% CI, 77.3–85.7), and those without these antibodies (87.9%; 95% CI, 85.6–89.9; $P < 0.001$) (Figure 1b).

The univariate associations of clinical, histologic, and immunologic factors with kidney allograft loss are shown in Table 2. In multivariable analysis the following independent determinants of kidney allograft loss were identified: glomerular filtration rate (hazard ratio [HR], 0.21; 95% CI, 0.16–0.29; $P < 0.001$), proteinuria (HR, 2.25; 95% CI, 1.85–2.73; $P < 0.001$), DSA and anti-AT1R antibody status (HR, 1.49; 95% CI, 1.07–2.06 for anti-AT1R antibodies alone; HR, 1.38; 95% CI, 1.05–1.84 for DSAs alone; and HR, 2.26; 95% CI, 1.52–3.36 for both antibodies; $P < 0.001$), glomerulitis and peritubular capillaritis (HR, 1.79; 95% CI, 1.32–2.43; $P < 0.001$), and interstitial fibrosis and tubular atrophy (HR, 1.29; 95% CI, 1.00–1.67; $P = 0.046$) (Table 3).

Kidney allograft injury

Kidney allograft injury according to anti-AT1R antibody status. Patients with anti-AT1R antibodies showed a higher incidence of histologic features of active antibody-mediated rejection at 1 year after transplantation as defined by the international classification for allograft rejection[34] (microvascular inflammation with sum of glomerulitis and peritubular capillaritis Banff scores > 1) compared with patients without anti-AT1R antibodies: 126 of 504 (25.0%) versus 173 of 1341 (12.9%), $P < 0.001$. Patients with anti-AT1R antibodies had a greater incidence of vascular rejection lesions, including microcirculation inflammation (incidence of glomerulitis of 147/504 [29.2%] vs. 277/1341 [20.7%], $P < 0.001$, and incidence of peritubular capillaritis of 145/504 [28.8%] vs. 232/1341 [17.3%], $P < 0.001$) and arterial inflammation (incidence of intimal arteritis of 56/504 [11.1%] vs. 53/1341 [4.0%], $P < 0.001$), compared with

Table 1 | Demographic and clinical characteristics according to the presence of circulating anti-AT1R antibodies

Characteristics	All patients (n = 1845)	AT1R-Ab negative (n = 1341)	AT1R-Ab positive (n = 504)	P
Recipient age, mean (SD), yr	48.6 (13.2)	49.3 (12.6)	46.9 (14.5)	0.001
Recipient female gender	744 (40.3)	497 (37.1)	247 (49.0)	<0.001
Recipient blood type				0.046
A	852 (46.2)	639 (47.7)	213 (42.3)	
B	162 (8.8)	122 (9.1)	40 (7.9)	
O	754 (40.9)	531 (39.6)	223 (44.2)	
AB	77 (4.1)	49 (3.6)	28 (5.6)	
Chronic kidney disease				0.160
Glomerulopathy	481 (26.1)	335 (25.0)	146 (29.0)	
Vascular nephropathy	141 (7.6)	108 (8.1)	33 (6.5)	
Chronic interstitial nephropathy	224 (12.1)	166 (12.4)	58 (11.5)	
Congenital nephropathy	355 (19.3)	269 (20.1)	86 (17.1)	
Diabetes	167 (9.1)	114 (8.4)	53 (10.5)	
Other	76 (4.1)	61 (4.5)	15 (3.0)	
Not determined	401 (21.7)	288 (21.5)	113 (22.4)	
Previous transplantation	334 (18.1)	216 (16.1)	118 (23.4)	<0.001
Preemptive transplantation	242 (13.1)	176 (13.1)	66 (13.1)	0.987
Time since dialysis, mean (SD), yr	4.9 (4.6)	4.9 (4.6)	4.9 (4.6)	0.852
Donor age, mean (SD), yr	51.5 (16.3)	52.1 (16.0)	50.0 (17.0)	0.015
Donor male gender	1008 (54.6)	715 (53.3)	293 (58.1)	0.064
Donor type				0.001
Living	336 (18.2)	220 (16.4)	116 (23.0)	
Deceased	1509 (81.8)	1121 (83.6)	388 (77.0)	
Donor serum creatinine, mean (SD), µmol/L	86.6 (51.2)	87.4 (54.1)	84.7 (42.4)	0.266
Cold ischemia time, mean (SD), h	17.7 (9.9)	18.0 (10.0)	16.8 (9.8)	0.017
HLA-A mismatch				0.713
0	451 (24.5)	328 (24.5)	123 (24.4)	
1	949 (51.4)	696 (51.9)	253 (50.2)	
2	445 (24.1)	317 (23.6)	128 (25.4)	
HLA-B mismatch				0.030
0	344 (18.6)	231 (17.2)	113 (22.4)	
1	894 (48.5)	667 (49.7)	227 (45.1)	
2	607 (32.9)	443 (33.1)	164 (32.5)	
HLA-DR mismatch				0.768
0	548 (29.7)	393 (29.3)	155 (30.7)	
1	967 (52.4)	704 (52.5)	263 (52.2)	
2	330 (17.9)	244 (18.2)	86 (17.1)	
HLA-DQ mismatch				0.959
0	482 (26.1)	350 (26.1)	132 (26.2)	
1	1011 (54.8)	733 (54.7)	278 (55.2)	
2	352 (19.1)	258 (19.2)	94 (18.6)	
Calculated panel reactive antibody				<0.001
0%	471 (25.5)	393 (29.3)	78 (15.5)	
1–24%	289 (15.7)	215 (16.0)	74 (14.7)	
25–49%	250 (13.5)	169 (12.6)	81 (16.1)	
50–84%	356 (19.3)	241 (18.0)	115 (22.8)	
85–100%	479 (26.0)	323 (24.1)	156 (30.9)	
Induction therapy				0.443
Thymoglobuline	1287 (69.8)	925 (69.0)	362 (71.8)	
Anti-CD25 therapy	554 (30.0)	413 (30.8)	141 (28.0)	
None	4 (0.22)	3 (0.22)	1 (0.20)	
Maintenance immunosuppression				
IMDPHi	1776 (96.3)	1296 (96.6)	480 (95.2)	0.156
Tacrolimus	1267 (68.7)	905 (67.5)	362 (71.8)	0.073
Cyclosporine	559 (30.3)	423 (31.5)	136 (27.0)	0.058
mTOR inhibitor	23 (1.2)	16 (1.2)	7 (1.4)	0.814
Steroids	1612 (87.4)	1161 (86.6)	451 (89.5)	0.094
Glomerular filtration rate, mean (SD), ml/min per 1.73 m^2	54.8 (20.9)	55.9 (21.5)	51.9 (19.0)	<0.001
Urine protein/creatinine ratio, mean (SD), g/g	0.34 (0.78)	0.28 (0.60)	0.52 (1.14)	<0.001
Post-transplant HLA DSA	467 (25.3)	334 (24.9)	133 (26.4)	0.514
All HLA DSA				
No. of specificities, mean (SD)	1.9 (1.3)	1.9 (1.2)	2.0 (1.5)	0.684
HLA class				0.817
I	96 (20.5)	71 (21.3)	25 (18.7)	

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Table 1 | (Continued)

Characteristics	All patients (n = 1845)	AT1R-Ab negative (n = 1341)	AT1R-Ab positive (n = 504)	P
II	207 (44.2)	146 (43.7)	61 (45.5)	
I+II	165 (35.3)	117 (35.0)	48 (35.8)	
Immunodominant HLA DSA[a]				
HLA class				0.373
I	144 (30.8)	107 (32.0)	37 (27.8)	
II	323 (69.2)	227 (68.0)	96 (72.2)	
MFI, mean (SD, range)	5177 (4434, 1103–20,746)	5055 (4481, 1103–20,746)	5486 (4314, 1215–18,755)	0.255
HLA DSA detectable before transplantation	342 (18.5)	233 (17.5)	109 (21.4)	0.053
Highest MFI, mean (SD, range)	3974 (4074, 1012–12,323)	3917 (4139, 1012–12,323)	4096 (3936, 1158–11,827)	0.669
Desensitization therapy	136 (7.4)	89 (6.6)	47 (9.3)	0.049

Values are n (%) unless otherwise defined. Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific antibody; HLA, human leukocyte antigen; IMPDHi, inosine-5′-monophosphate dehydrogenase inhibitor; MFI, mean fluorescence intensity; mTOR, mammalian target of rapamycin.
[a]The highest MFI value toward a donor-specific allele was considered to be the immunodominant DSA.

patients without anti-AT1R antibodies (Table 4). Higher levels of circulating anti-AT1R antibodies were associated with increased risk of glomerulitis, peritubular capillaritis, and intimal arteritis (Figure 2). Stratified analysis according to the type of allograft biopsy showed the consistency of these associations in patients undergoing clinical indication biopsy within the first year after transplantation and in patients undergoing protocol biopsy 1 year after transplantation (Table S1).

Stratified analysis according to DSA status. Stratified analysis according to DSA status revealed the consistency of the associations between anti-AT1R antibodies and allograft injury in patients without DSAs and in those with DSAs (Supplementary Table S2). The incidence of histologic features of active antibody-mediated rejection at 1 year after transplantation was increased in patients with anti-AT1R antibodies compared with patients without anti-AT1R antibodies among patients without DSAs (51/371 [13.7%] vs. 26/1007 [2.6%], $P < 0.001$) and in patients with DSAs (75/133 [56.4%] vs. 147/334 [44.0%], $P = 0.016$). In patients without DSAs (n = 1378), those with anti-AT1R antibodies (n = 371) showed greater incidence of microvascular (incidence of glomerulitis of 68/371 [18.3%] vs. 114/1007 [11.3%], $P = 0.001$, and incidence of peritubular capillaritis of 64/371 [17.3%] vs. 73/1007 [7.3%], $P < 0.001$) and arterial inflammation (incidence of intimal arteritis of 40/371 [10.8%] vs. 34/1007 [3.4%], $P < 0.001$) compared with patients without anti-AT1R antibodies (n = 1007). Among patients with DSAs (n = 467), those with anti-AT1R antibodies (n = 133) showed greater incidence of microvascular (incidence of glomerulitis of 79/133 [59.4%] vs. 163/334 [48.8%], $P = 0.039$, and incidence of peritubular capillaritis of 81/133 [60.9%] vs. 159/334 [47.6%], $P = 0.009$) and arterial inflammation (incidence of intimal arteritis of 16/133 [12.0%] vs. 19/334 [5.7%], $P = 0.019$) compared with patients without anti-AT1R antibodies (n = 334).

Anti-AT1R antibody–associated rejection phenotype

Among 299 patients (16.2%) with histologic features of active antibody-mediated rejection (microvascular inflammation with sum of glomerulitis and peritubular capillaritis Banff scores > 1) (Table 5), the detection of anti-AT1R antibodies identified 51 of 77 patients (66.2%) as having active antibody-mediated rejection in patients without DSAs. Among them, 36 patients (70.6%) had pre-existing anti-AT1R antibodies detected in the sera collected at the time of transplantation, and 15 patients (29.4%) were found to have *de novo* anti-AT1R antibodies. Patients with pre-existing anti-AT1R antibodies (n = 36) showed increased levels of anti-AT1R antibodies between the time of transplantation and the time of ABMR diagnosis ($P < 0.001$) (Supplementary Figure S2). Q5

Compared with patients with prototypical DSA-mediated rejection (HLA-DSA+, AT1R-Ab–, n = 147), patients with anti-AT1R antibody–associated rejection (HLA-DSA–, AT1R-Ab+, n = 51) exhibited greater prevalence of hypertension (46/147 [31.3%] vs. 32/51 [62.7%], $P < 0.001$), similar glomerular filtration rate (32.5 [20.7] vs. 34.2 [18.9] ml/min per 1.73 m^2, $P = 0.61$) and proteinuria level (1.08 [1.60] vs. 0.93 [1.57], $P = 0.56$), and similar cold ischemia time (18.3 [9.5] vs. 17.4 [10.4], $P = 0.57$). Antibody-mediated rejection was diagnosed at a median time since transplantation of 11.3 months (interquartile range, 2.8–12.0) in patients with anti-AT1R antibody–associated rejection versus 5.6 months (interquartile range, 2.8–11.3) in patients with DSA-mediated rejection ($P = 0.11$). Patients with anti-AT1R antibody–associated rejection showed greater prevalence of arterial inflammation (18/147 [12.2%] vs. 22/51 [43.1%], $P < 0.001$), decreased prevalence of complement deposition in peritubular capillaries (73/147 [49.7%] vs. 7/51 [13.7%], $P < 0.001$), and increased endothelial activation in allografts reflected by higher levels of expression of endothelial-associated transcripts (ENDATs) ($P = 0.013$) compared with patients with prototypical DSA-mediated rejection. Figure 3 illustrates the pattern of complement-independent antibody-mediated vascular rejection observed in kidney allografts of patients with circulating anti-AT1R antibodies.

DISCUSSION

In a prospective cohort of 1845 kidney transplant recipients, we observed that the presence of circulating non-HLA agonistic anti-AT1R antibodies was associated with increased incidence of antibody-mediated allograft rejection

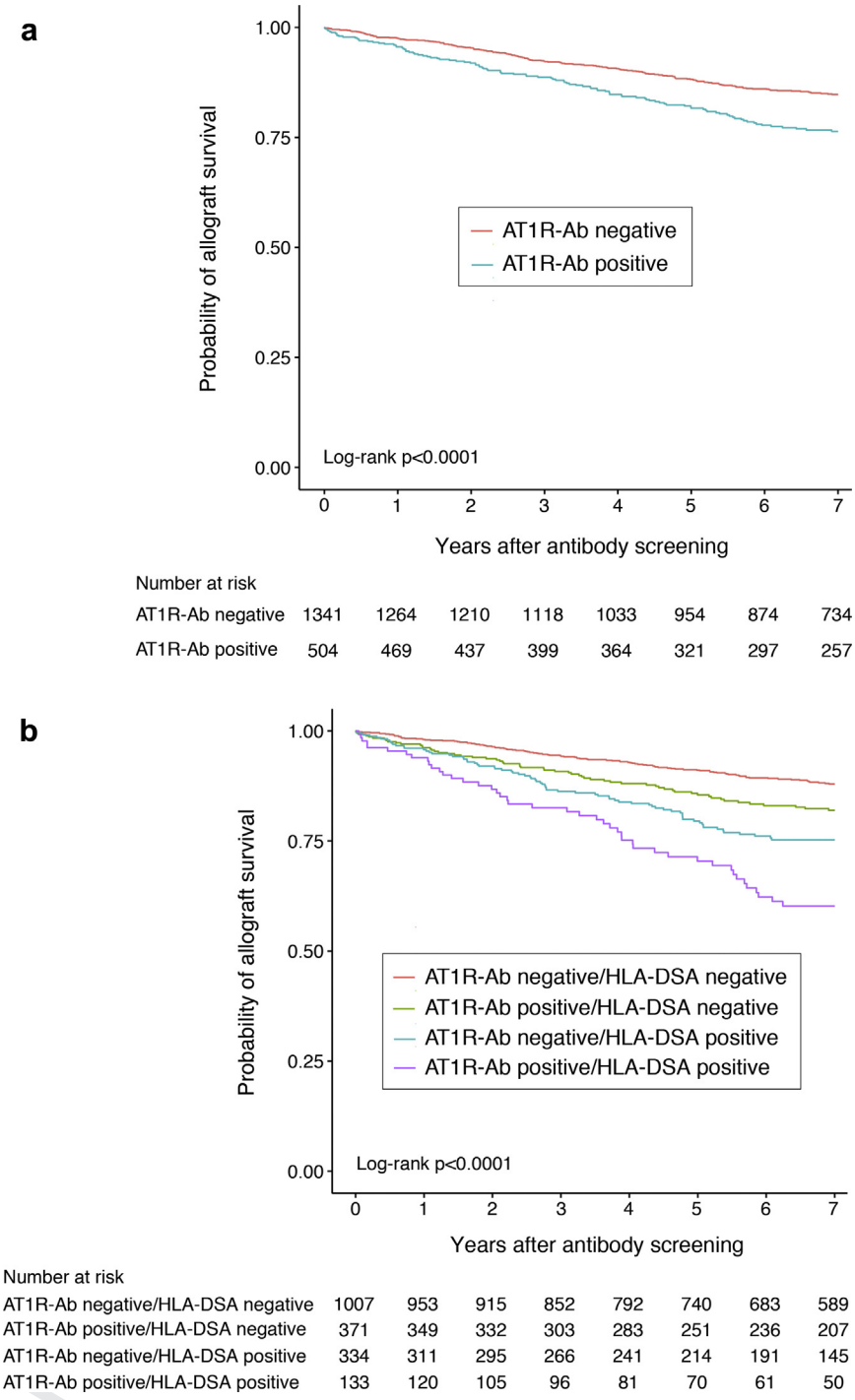

Figure 1 | Kaplan-Meier curves for death-censored kidney allograft survival after antibody screening according to post-transplant circulating anti-AT1R receptor antibody status. Data are based on 1845 kidney transplant recipients undergoing systematic screening for circulating DSAs and anti-AT1R antibodies within the first year after transplantation. (**a**) Kaplan-Meier curves for death-censored kidney allograft survival on the basis of the presence of post-transplant anti-AT1R antibodies. (**b**) Kaplan-Meier curves for death-censored kidney allograft survival according to post-transplant anti-AT1R antibody status and post-transplant DSA status. Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific anti-HLA antibody; HLA, human leukocyte antigen.

at 1 year after transplantation and decreased long-term allograft survival. These increased risks of allograft rejection and allograft loss associated with anti-AT1R antibodies were present in patients without DSAs and in those with DSAs, with both a main effect and a synergistic effect of these antibodies on allograft injury. Anti-AT1R antibody–associated rejection was characterized by hypertension; active vascular lesions (microcirculation inflammation and arterial inflammation); increased expression of ENDATs, which revealed antibody interaction with the vascular endothelium[34]; and low levels of

Table 2 | Determinants of kidney allograft loss assessed at the time of antibody screening: univariate analysis

	Number of patients	Number of events	HR	95% CI	P
		Clinical			
Recipient age, per 1-year increment	1845	285	0.99	0.98–1.00	0.117
Recipient gender					
Male	1101	178	1		
Female	744	107	0.88	0.69–1.12	0.309
Type of allograft biopsy					
One-year biopsy	1541	189	1		
Clinical indication biopsy in the first year	304	96	2.68	2.09–3.42	<0.001
GFR (log transformed), per 1-ml/min per 1.73 m^2 increment	1845	285	0.14	0.11–0.18	<0.001
Proteinuria (square root value), per 1-g/g increment	1845	285	3.31	2.80–3.92	<0.001
Previous transplantation					
No	1511	210	1		
Yes	334	75	1.75	1.34–2.27	<0.001
Preemptive transplantation					
No	1603	277	1		
Yes	242	8	0.16	0.08–0.33	<0.001
Time since dialysis, per 1-year increment	1603	277	1.03	1.00–1.05	0.031
Donor age, per 1-year increment	1845	285	1.01	1.00–1.02	0.002
Donor gender					
Male	1008	150	1		
Female	837	135	1.09	0.87–1.38	0.458
Donor type					
Living	336	33	1		
Deceased	1509	252	1.81	1.26–2.61	0.001
Cold ischemia time, per 1-hour increment	1845	285	1.02	1.01–1.04	<0.001
No. of HLA-A/-B/-DR/-DQ mismatch, per 1-unit increment	1845	285	1.02	0.92–1.12	0.749
Pretransplant DSA					
No	1503	200	1		
Yes	342	85	2.06	1.60–2.65	<0.001
		Immunology			
AT1R and HLA antibody status					<0.001
None	1007	106	1		
Anti-AT1R antibody	371	60	1.59	1.16–2.18	
DSA	334	74	2.27	1.69–3.05	
Anti-AT1R and DSA	133	45	3.88	2.74–5.50	
		Histology			
Glomerulitis + peritubular capillaritis					
Banff score ≤ 1	1546	186	1		
Banff score > 1	299	99	3.13	2.46–4.00	<0.001
Interstitial inflammation + tubulitis					
Banff score ≤ 1	1528	211	1		
Banff score > 1	317	74	1.75	1.34–2.28	<0.001
Intimal arteritis					
Banff score 0	1736	254	1		
Banff score > 0	109	31	2.16	1.53–3.08	<0.001
Chronic allograft glomerulopathy					
Banff score 0	1755	260	1		
Banff score > 0	90	25	2.13	1.41–3.22	<0.001
Interstitial fibrosis and tubular atrophy					
Banff score ≤ 1	1354	178	1		
Banff score > 1	491	107	1.83	1.44–2.33	<0.001
Arteriosclerosis					
Banff score ≤ 1	1183	166	1		
Banff score > 1	662	119	1.40	1.10–1.76	0.006
Arteriolar hyalinosis					
Banff score ≤ 1	1537	217	1		
Banff score > 1	308	68	1.71	1.30–2.25	<0.001
C4d deposition in peritubular capillaries					
Banff score 0	1660	225	1		
Banff score > 0	185	60	2.68	2.02–3.57	<0.001

AT1R, angiotensin II type 1; CI, confidence interval; DSA, donor-specific anti-HLA antibody; GFR, glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio.

Table 3 | Determinants of kidney allograft loss assessed at the time of antibody screening: multivariable model

	Number of patients	Number of events	HR	95% CI	P
GFR (log transformed), per 1-ml/min per 1.73 m^2 increment	1845	285	0.21	0.16–0.29	<0.001
Proteinuria (square root value), per 1-g/g increment	1845	285	2.25	1.85–2.73	<0.001
AT1R and HLA antibody status					<0.001
None	1007	106	1		
Anti-AT1R antibody	371	60	1.49	1.07–2.06	
DSA	334	74	1.38	1.05–1.84	
Anti-AT1R and DSA	133	45	2.26	1.52–3.36	
Glomerulitis + peritubular capillaritis					
Banff score ≤ 1	1546	186	1		
Banff score > 1	299	99	1.79	1.32–2.43	<0.001
Interstitial fibrosis and tubular atrophy					
Banff score ≤ 1	1354	178	1		
Banff score > 1	491	107	1.29	1.00–1.67	0.046

AT1R, angiotensin II type 1; CI, confidence interval; DSA, donor-specific anti-HLA antibody; GFR, glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio.

complement deposition in allograft capillaries. The detection of anti-AT1R antibodies identified 51 patients (66.2%) as having antibody-mediated rejection among 77 patients with histologic features of antibody-mediated rejection, in whom a definitive diagnosis could not be reached because of the lack of serologic evidence of DSAs.

The broadest evidence suggesting a role of non-HLA immunity in transplantation was provided by a large registry study that observed a significant association between the presence of lymphocytotoxic antibodies detected before transplantation and allograft survival in kidney recipients from HLA-identical sibling donors, although the nature of these antibodies and their pathogenic expression in allografts were not evaluated.[38] Description of antibody-mediated AT1R activation in vascular cells and proof of concept in animal models emphasized the vasculature as the critical interface between the recipient immune system and the transplanted organ even in the absence of classic complement activation features.[10] Several studies have evaluated the potential role of agonistic antibodies that target AT1R and have reported conflicting results. These studies were limited by small sample size, inclusion of selected populations, lack of systematic allograft biopsy performance for assessing correlations between immunologic status and injury phenotype, and/or lack of evaluation of concomitant DSA status using a sensitive technique.[10–22,24,26–31] Therefore, no definitive conclusion has been reached regarding the clinical value of anti-AT1R antibody screening as part of the immunologic assessment of transplant recipients, which to date remains entirely based on the HLA system.[7,33]

Our data support that post-transplant non-HLA agonistic anti-AT1R antibodies may contribute to a specific type of kidney allograft rejection characterized by vascular injury with microvascular and arterial inflammation, endothelial activation, and lack of complement activation. These findings are consistent with previous reports of kidney transplant recipients with anti-AT1R antibody–associated allograft injury who often demonstrated involvement of allograft arteries and lack of complement deposition.[10] Moreover, we observed a biologic gradient between the level of circulating anti-AT1R

Table 4 | Kidney allograft injury according to the presence of circulating anti-AT1R antibodies

	Overall (n = 1845)	AT1R-Ab negative (n = 1341)	AT1R-Ab positive (n = 504)	P
Type of allograft biopsy				0.262
Clinical indication biopsy	304 (16.5)	213 (15.9)	91 (18.1)	
One-year protocol biopsy	1541 (83.5)	1128 (84.1)	413 (81.9)	
Glomerulitis	424 (23.0)	277 (20.7)	147 (29.2)	<0.001
Peritubular capillaritis	377 (20.4)	232 (17.3)	145 (28.8)	<0.001
Interstitial inflammation	393 (21.3)	281 (21.0)	112 (22.2)	0.553
Tubulitis	381 (20.7)	269 (20.1)	112 (22.2)	0.307
Intimal arteritis	109 (5.9)	53 (4.0)	56 (11.1)	<0.001
Chronic allograft glomerulopathy	90 (4.9)	58 (4.3)	32 (6.4)	0.072
Interstitial fibrosis and tubular atrophy	1095 (59.4)	789 (58.8)	306 (60.7)	0.464
Arteriosclerosis	1187 (64.3)	872 (65.0)	315 (62.5)	0.313
Arteriolar hyalinosis	1084 (58.8)	794 (59.2)	290 (57.5)	0.516
C4d deposition	185 (10.0)	125 (9.3)	60 (11.9)	0.100
Pathologic features of ABMR with DSAs	222 (12.0)	147 (11.0)	75 (14.8)	0.021
Pathologic features of ABMR without DSAs	77 (4.2)	26 (1.9)	51 (10.1)	<0.001
T cell–mediated rejection	182 (9.9)	128 (9.6)	54 (10.7)	0.453

Values are n (%). Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-AT1R antibodies and kidney allograft histology within the first year after transplantation. Histologic assessment of kidney allograft injury was performed according to the Banff classification for allograft rejection. Each individual lesion was considered present (Banff score of 1, 2, or 3) or absent (Banff score of 0). Pathologic features of active antibody-mediated rejection were defined by microcirculation inflammation with the sum of glomerulitis and peritubular capillaritis Banff scores > 1 according to the Banff classification. Ab, antibody; ABMR, active antibody-mediated rejection; AT1R, angiotensin II type 1 receptor; DSA, donor-specific anti-HLA antibody; HLA, human leukocyte antigen.

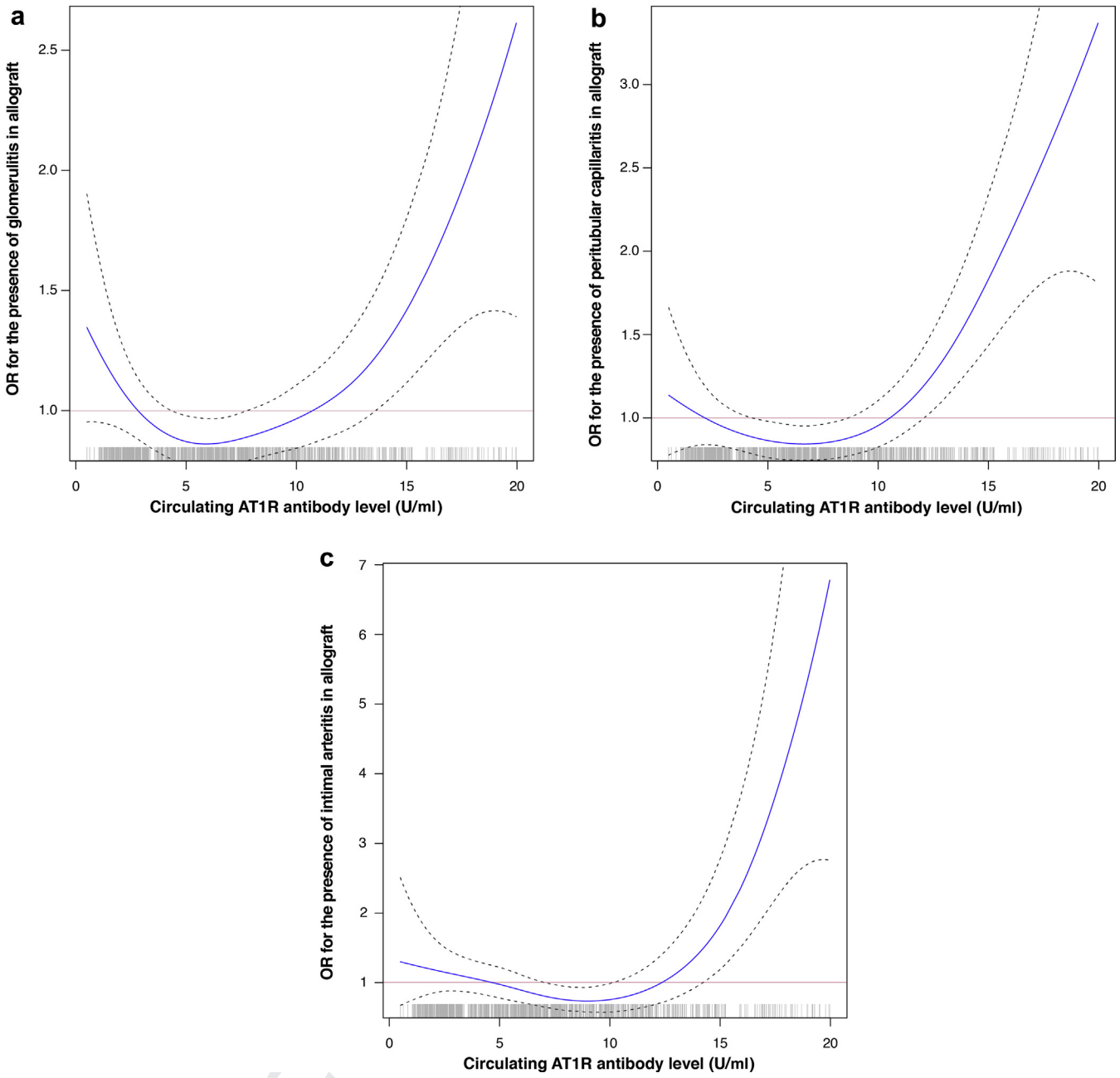

Figure 2 | Risk of occurrence of microcirculation and arterial injury according to post-transplant circulating anti-AT1R antibody level. Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-AT1R antibodies and kidney allograft histology within the first year after transplantation. The blue curve represents the relationship between circulating anti-AT1R antibody level and the risk of occurrence of glomerulitis (**a**), peritubular capillaritis (**b**), and intimal arteritis (**c**) in allografts on the basis of penalized splines. Dashed lines denote 95% confidence intervals. The gray bars represent individual anti-AT1R antibody levels. The spline curves were truncated at anti-AT1R antibody level of 20 U/ml given the low number of patients above this value (74/1845, 4.0%). Significant odds ratios are observed from AT1R antibody levels between 10 and 15 U/ml for glomerulitis (**a**), peritubular capillaritis (**b**), and intimal arteritis (**c**), where the lower limit of the 95% confidence intervals are above an odds ratio of 1 (red line). AT1R, angiotensin II type 1 receptor; OR, odds ratio.

antibodies and allograft antibody–mediated injury and allograft loss, with exposure to higher levels of anti-AT1R antibodies associated with greater prevalence of concomitant microcirculation and arterial injuries and greater incidence of allograft loss, reinforcing the potential causal role of these antibodies as mediators of allograft rejection. Biologic plausibility is supported by the current knowledge regarding the direct endothelial pathogenicity of agonistic anti-AT1R antibodies attributed to the AT1R activation process in different disease contexts ranging from allograft rejection with vascular involvement to severe systemic autoimmune vasculopathies.[39–41]

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Table 5 | Kidney allograft injury according to the presence of post-transplant circulating anti-AT1R antibodies and circulating DSAs in patients with histologic features of active antibody-mediated rejection

	Overall (n = 299)	AT1R-Ab− HLA-DSA− (n = 26)	AT1R-Ab+ HLA-DSA− (n = 51)	AT1R-Ab− HLA-DSA+ (n = 147)	AT1R-Ab+ HLA-DSA+ (n = 75)	*P*
Type of allograft biopsy						0.001
Clinical indication biopsy	175 (58.5)	7 (26.9)	29 (56.9)	84 (57.1)	55 (73.3)	
One-year protocol biopsy	124 (41.5)	19 (73.1)	22 (43.1)	63 (42.9)	20 (26.7)	
Glomerulitis	285 (95.3)	22 (84.6)	49 (96.1)	141 (95.9)	73 (97.3)	0.094
Peritubular capillaritis	285 (95.3)	20 (76.9)	49 (96.1)	141 (95.9)	75 (100)	<0.001
Interstitial inflammation	117 (39.1)	12 (46.2)	21 (41.2)	57 (38.8)	27 (36.0)	0.805
Tubulitis	102 (34.1)	8 (30.8)	21 (41.2)	49 (33.3)	21 (28.0)	0.484
Intimal arteritis	59 (19.7)	3 (11.5)	22 (43.1)	18 (12.2)	16 (21.3)	<0.001
Chronic allograft glomerulopathy	50 (16.7)	2 (7.7)	6 (11.8)	26 (17.7)	16 (21.3)	0.338
Interstitial fibrosis and tubular atrophy	219 (73.2)	14 (53.9)	41 (80.4)	108 (73.5)	56 (74.7)	0.111
Arteriosclerosis	202 (67.6)	17 (65.4)	31 (60.8)	100 (68.0)	54 (72.0)	0.598
Arteriolar hyalinosis	173 (57.9)	15 (57.7)	32 (62.8)	88 (59.9)	38 (50.7)	0.509
C4d deposition	121 (40.5)	2 (7.7)	7 (13.7)	73 (49.7)	39 (52.0)	<0.001

Values are n (%). Data are based on 1845 kidney allograft biopsies performed within the first year after transplantation, in which 299 (16.2%) showed histologic features of active antibody-mediated rejection (sum of glomerulitis and peritubular capillaritis Banff scores > 1). Histologic assessment of kidney allograft injury was performed according to the Banff classification for allograft rejection. Each individual lesion was considered present (Banff score of 1, 2, or 3) or absent (Banff score of 0). Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific anti-HLA antibody; HLA, human leukocyte antigen.

One of the major limitations faced by current diagnostic standards in organ transplantation is represented by the diagnostic uncertainty in patients showing histologic features of antibody-mediated rejection without evidence for DSAs, with discordances between clinicians' diagnoses and reference standard in nearly 50% of cases.[42] This diagnostic uncertainty leads to unresolved therapeutic questions because current approaches in patients with antibody-mediated rejection are directed toward removal of circulating DSAs, blockade of their effects, and reduction of their production.[43] The identification of anti-AT1R antibodies as a potential mediator of allograft rejection and loss, independent of the presence of DSAs, has important implications for the clinical management of transplant recipients by enabling the development of specific therapeutic strategies. Future studies should evaluate the efficacy of current antibody-targeting therapies, such as plasma exchange and i.v. immune globulins, which are the standard of care treatment for anti-HLA antibody-mediated allograft rejection.[43] Moreover, our study paves the way for future therapeutic approaches; a selective blockade of AT1Rs using sartans could alleviate allograft vascular injury and prevent long-term allograft loss mediated by agonistic anti-AT1R antibodies. Reports have actually indicated good outcomes using AT1R blockers for treating kidney transplant recipients with anti-AT1R antibody–associated rejection in terms of clinical and histologic course[44] and allograft survival,[10] as well as for preventing kidney allograft rejection in recipients with high levels of circulating anti-AT1R antibodies.[45] However, no clinical trial has been conducted to date to demonstrate the benefit of such approaches. In addition, AT1R blockers may exert a clinically relevant immunomodulatory effect exemplified by a reduction in interferon-γ generation by T cells as demonstrated *in vitro* and *in vivo*.[46] Finally, the low level of complement activation observed in patients with anti-AT1R antibody–mediated rejection suggests that strategies based on complement inhibition, such as terminal C5 or proximal C1 component blockade that have been used in patients with DSAs,[47] may not be relevant in patients with anti-AT1R antibody–mediated rejection. Potential therapeutic advances in anti-AT1R antibody–mediated vascular rejection may offer new opportunities for the management of systemic autoimmune vasculopathies related to AT1R activation.

Our study has some limitations. The assessment of anti-AT1R antibody status and allograft injury was limited to the first year after transplantation to limit the confounding background of previous allograft injuries. One has to consider potential interlaboratory variability of anti-AT1R antibody measurement, which remains to be investigated, although most studies have reported a comparable cut-point for anti-AT1R antibody positivity. Additionally, there might be residual confounding in the association between anti-AT1R antibodies and allograft survival and injury represented by other non-HLA antibodies. Finally, although our data indicate a clinical value of anti-AT1R antibody assessment in patients with pathologic features of antibody-mediated rejection (with or without detectable DSAs), specific studies dedicated to the evaluation of cost-effectiveness, including the consideration of resource-limited settings and the cost related to allograft biopsies triggered by anti-AT1R antibody positivity, are needed to substantiate the utility of a systematic monitoring of anti-AT1R antibodies as part of the standard of care management of kidney transplant recipients. Nevertheless, given the strong phenotypic association with C4d-negative ABMR, one should consider anti-AT1R antibodies as an important diagnostic tool.

In conclusion, our study showed the clinical relevance of the screening for circulating functional non-HLA anti-AT1R antibodies in addition to the current approach for immunologic assessment of kidney transplant recipients by identifying patients at high risk of allograft rejection and allograft loss independent of the HLA system. We completed the

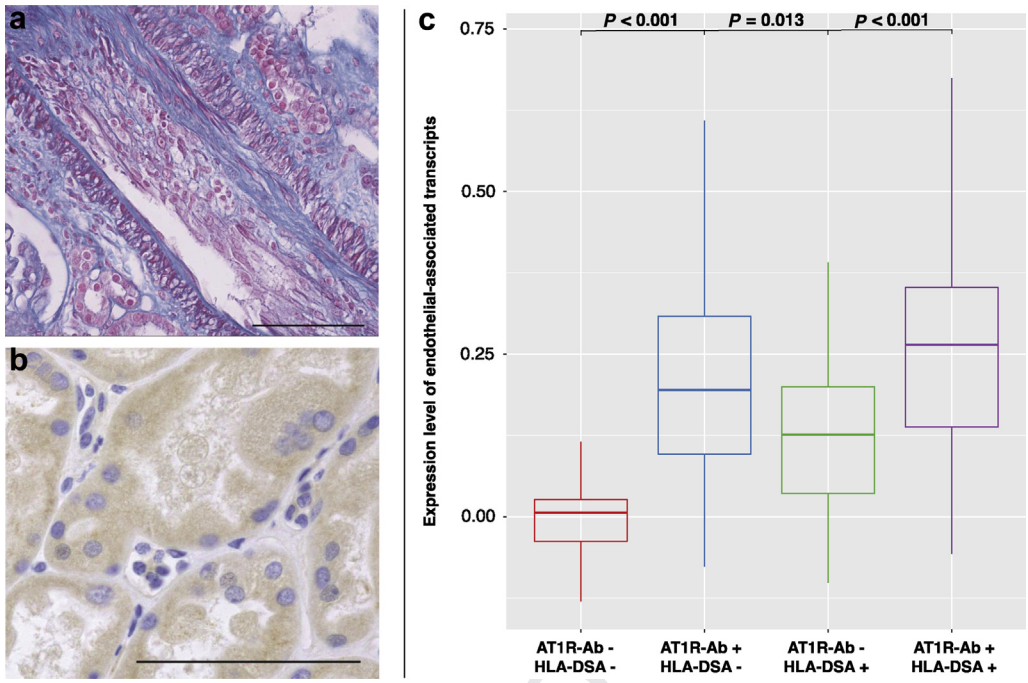

Figure 3 | Complement-independent antibody-mediated vascular kidney allograft rejection in patients with circulating anti-AT1R antibodies. Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-AT1R antibodies and kidney allograft histology within the first year after transplantation, in whom 299 (16.2%) showed histologic features of active antibody-mediated rejection according to the Banff classification for allograft rejection (microcirculation inflammation with sum of glomerulitis and peritubular capillaritis Banff scores > 1). Among patients with histologic features of active antibody-mediated rejection, 51 (17.0%) had anti-AT1R antibodies, 147 (49.2%) had DSAs, 75 (25.1%) had both antibodies, and 26 (8.7%) had no antibody. Anti-AT1R antibody–associated rejection was characterized by vascular inflammation with microvascular and arterial inflammation, endothelial activation reflected by high levels of expression of endothelial-associated transcripts, and lack of complement deposition in allograft capillaries. (**a**) Severe intimal arteritis in a patient with anti-AT1R antibody–associated rejection characterized by subendothelial infiltration of an interlobular artery by inflammatory cells. Masson's trichrome stain, original magnification ×200, bar = 200 μm. (**b**) Peritubular capillaritis and lack of complement fraction C4d deposition in peritubular capillaries in the same patient with anti-AT1R antibody–associated rejection. Immunoperoxidase, original magnification ×400, bar = 200 μm. (**c**) Expression levels of endothelial-associated transcripts (ENDATs) in patients with features of active antibody-mediated rejection according to anti-AT1R antibody and DSA status. Expression levels of ENDATs were measured using microarray. ENDATs represent a pathogenesis-based transcript set that reflects endothelial activation and indicates current/recent antibody interaction with the vascular endothelium. Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific anti-HLA antibody; HLA, human leukocyte antigen. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

current understanding of autoimmune endothelial injury related to anti-AT1R autoantibodies, including systemic vascular disorders such as preeclampsia and scleroderma, by identifying a new pattern of allograft vascular rejection associated with anti-AT1R antibodies in kidney transplant recipients. Recognition of complement-independent anti-AT1R antibody–mediated vascular rejection could lead to the development of new treatment strategies targeting circulating antibodies and AT1Rs to improve allograft survival, in particular in forms of allograft rejection without evidence for HLA antibody involvement, for which no therapeutic strategy has been defined. The clinical relevance of anti-AT1R antibodies might be extended beyond renal transplantation to heart transplantation and other solid organs.

METHODS

Participants and study design

We prospectively enrolled all consecutive patients who underwent ABO blood group compatible kidney transplantation in Necker Hospital and Saint-Louis Hospital (Paris, France) between January 1, 2008 and December 31, 2012 (n = 1957) (ClinicalTrials.gov, NCT03466775).

These kidney recipients underwent allograft evaluation including assessment of allograft function, circulating DSAs and anti-AT1R antibodies, and histologic parameters measured on allograft biopsy within the first year after transplantation. Allograft evaluation was performed at the time of the first episode of biopsy-proven allograft rejection occurring in the first year after transplantation or by protocol at 1 year after transplantation in patients without any rejection episodes diagnosed in the first year after transplantation. We excluded patients receiving multiorgan transplantation (n = 19), patients without allograft biopsy performed within the first year after transplantation (n = 56), patients with inadequate allograft biopsy according to the Banff classification for allograft rejection[48] (n = 21), and patients without available serum for the assessment of anti-AT1R antibodies (n = 10) or DSAs (n = 6). Patients were followed annually up until December 31, 2017.

The transplantation allocation system followed the rules of the French national agency for organ procurement (Agence de la Biomédecine). All transplants were performed with negative standard

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National Institutes of Health and antihuman globulin T and B cell cytotoxicity crossmatches on serum obtained at the time of transplantation. All patients provided written informed consent.

Clinical data

The clinical data on donors and recipients were extracted from the DIVAT database (www.divat.fr). Coding was used to ensure strict donor and recipient anonymity. The data were entered into the database in real time or at each transplant anniversary. This database is approved by the National French Commission for Bioinformatics Data and Patient Liberty (Commission Nationale de l'Informatique et des Libertés registration no. 1016618; validated June 8, 2004). The data were retrieved from the database on December 31, 2017. Renal function was assessed by the estimated glomerular filtration rate with the abbreviated Modification of Diet in Renal Disease formula.[49] Hypertension was defined by a systolic blood pressure $\geq$ 140 mm Hg and/or a diastolic blood pressure $\geq$ 90 mm Hg.

Detection of anti-AT1R antibodies and DSAs

All patients were tested for the presence of circulating anti-AT1R antibodies using a quantitative ELISA (Celltrend, Luckenwalde, Germany). A value of 10 U/ml was considered the cut-off point for anti-AT1R antibody positivity after examining prior studies of its association with allograft rejection and loss[12,14–17,20–22,24,28,31]; this cut-off point also represented the third quartile value in the distribution of anti-AT1R antibody values in the entire study population (Supplementary Figure S3).

The presence of circulating anti-HLA-A, -B, -Cw, -DR, -DQ, and -DP DSAs was assessed using single-antigen flow bead assays (One Lambda, Inc., Canoga Park, CA) that were used on a Luminex platform (Regional Histocompatibility Laboratory, Paris, France). All beads showing a normalized mean fluorescence intensity $>$ 1000 were considered positive. HLA typing of all kidney transplant donors and recipients was performed by molecular biology (Innolipa HLA Typing Kit; Innogenetics, Gent, Belgium).

Histologic and immunochemical phenotyping of kidney allograft biopsies

All allograft biopsy specimens (304 allograft biopsies performed for clinical indication, with a median time between transplantation and allograft biopsy of 3.8 months [interquartile range, 2.3–7.3] and 1541 protocol biopsies performed at 1 year after transplantation) were scored and graded from 0 to 3 according to the updated Banff criteria[34] by a trained pathologist. The pathologist examined the following histologic factors: glomerulitis, peritubular capillaritis, mononuclear cell interstitial inflammation, tubulitis, intimal arteritis, chronic allograft glomerulopathy, interstitial fibrosis and tubular atrophy, arteriolar hyaline thickening, and vascular fibrous intimal thickening. The pathologist was blinded to the clinical data and to the original pathologic report. Complement split-product C4d staining was performed by immunochemical analysis on paraffin sections using polyclonal rabbit antihuman C4d antibodies (Biomedica Gruppe, Vienna, Austria).

Allograft diagnoses were defined according to the Banff classification. Active ABMR was defined by (1) histologic evidence of acute tissue injury, including 1 or more of the following: glomerulitis score $>$ 0 and/or peritubular capillaritis score $>$ 0 (in the absence of recurrent or *de novo* glomerulonephritis and with glomerulitis score $\geq$ 1 in case of acute T cell–mediated rejection, borderline infiltrate, or infection), intimal arteritis score $>$ 0, acute thrombotic microangiopathy in the absence of any other cause, acute tubular injury in the absence of any other apparent cause; (2) evidence of current/recent antibody interaction with vascular endothelium, including 1 or more of the following: C4d score $>$ 0, glomerulitis + peritubular capillaritis scores $\geq$ 2; and (3) serologic evidence of DSAs and/or anti-AT1R antibodies.

RNA extraction and gene expression analysis in kidney allograft biopsies

Kidney allograft biopsies showing pathologic features of active antibody-mediated rejection, as defined by the Banff classification for allograft rejection (microcirculation inflammation with sum of glomerulitis and peritubular capillaritis Banff scores $>$ 1)[34] were processed for microarray analysis as previously described.[50] One biopsy sample was immediately placed in a dry tube and stored at –80°C. RNA extraction, labeling, and hybridization to HG-U219 GeneChip arrays (Affymetrix, Santa Clara, CA) were performed according to the manufacturer's protocols (www.affymetrix.com). The microarrays were scanned using the Gene Array Scanner (Affymetrix) and processed with GeneChip Operating Software version 1.4.0 (Affymetrix), and robust multiarray averaging was used to normalize the microarrays. The microarray data files were processed using robust multiarray analysis in Bioconductor. Q6

We measured and compared intragraft expression levels of ENDATs according to anti-AT1R antibodies and anti-HLA DSA status. ENDATs represent a pathogenesis-based transcript set that reflects endothelial activation.[51] The details of the probe set can be found in Supplementary Table S3.

Outcomes

The primary outcomes were kidney allograft loss and the incidence of biopsy-proven allograft rejection at 1 year post-transplantation. Kidney allograft loss was defined as the patient's return to chronic dialysis or preemptive subsequent kidney transplant. Allograft rejection episodes were diagnosed according to the Banff classification for allograft rejection.[34] Secondary outcomes included allograft injury phenotype based on histologic allograft elementary lesions defined by the Banff classification and ENDAT expression measured in kidney allograft biopsies showing histologic features of active antibody-mediated rejection (sum of glomerulitis and peritubular capillaritis Banff scores $>$ 1).

Statistical analysis

Continuous variables were described using means with SDs unless otherwise stated. We compared means and proportions using Student's t test and χ^2 test, respectively (or Mann-Whitney U test and Fisher's exact test, respectively, if appropriate). Survival was analyzed from the time of post-transplant allograft biopsy to a maximum of 7 years after allograft evaluation, with kidney allograft loss as the event of interest. In case of death with a functioning allograft, allograft survival was censored at time of death. Rates of kidney allograft survival were plotted on Kaplan-Meier curves and compared according to anti-AT1R antibodies and DSA status with the use of the log-rank test. Cox proportional hazards models were used to quantify HRs and 95% CIs for time to kidney allograft loss. The association of clinical, histologic, and immunologic parameters evaluated at the time of allograft evaluation with allograft loss was assessed in separate univariate and multivariable Cox regression analyses. The best transformation for continuous variables was determined using fractional polynomial method. A *P* value threshold of 0.20 for entering variables into the multivariable model was used.

The factors identified in these analyses were thereafter included in a final multivariable model with stepwise backward elimination. The proportional hazards assumption of the Cox model was verified with the log-graphic method.

We used penalized splines to represent the relationship between anti-AT1R antibody levels and the odds ratio for the presence of glomerulitis, peritubular capillaritis, and intimal arteritis and the HR for the occurrence of allograft loss. Biologic plausibility (that is, monotonicity) was considered, along with Akaike's information criterion, in selecting the optimal degrees of freedom.[52]

All tests were 2-sided, and except in the univariate analyses, $P < 0.05$ was regarded as statistically significant. All statistical analyses were performed using R version 3.4.3 (R Development Core Team, Vienna, Austria).

DISCLOSURE

All the authors declared no competing interests.

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This study was approved by Institutional Review Boards of Necker and Saint-Louis Hospitals. The DIVAT database network has been approved by the National French Commission for bioinformatics data and patient liberty: CNIL, registration number: 1016618, validated 8th June 2004.

AUTHOR CONTRIBUTIONS

PFH and DD are co–senior author. C Lefaucheur, AL, and DD conceived and designed the study. C Lefaucheur, DV, YB, DP, AL, and DD drafted the manuscript. C Lefaucheur, DV, YB, AP, OA, JPDVH, JLT, PH, AL, DG, C Legendre, and DD analyzed and interpreted the data. AP prepared the serum samples. JPDVH reported pathology results. All authors were involved in revising the article and approved the final version of the manuscript. All authors had full access to the data and made the decision collectively to submit the manuscript for publication.

SUPPLEMENTARY MATERIAL

Table S1. Kidney allograft injury at the time of antibody screening according to the presence of circulating anti-angiotensin II type 1 receptor antibodies and stratified on the basis of allograft biopsy indication.

Table S2. Kidney allograft injury at the time of antibody screening according to the presence of circulating anti-angiotensin II type 1 receptor antibodies and stratified on the basis of donor-specific anti-HLA antibody status.

Table S3. Endothelial-associated transcript (ENDAT) gene list with their corresponding probe set.

Figure S1. Risk of kidney allograft loss according to post-transplant circulating anti-angiotensin II type 1 receptor antibody level.

Figure S2. Distribution of pretransplant and post-transplant circulating anti-angiotensin II type 1 receptor antibody levels in patients with pre-existing anti-AT1R antibody–associated rejection without DSAs.

Figure S3. Distribution of post-transplant circulating anti-angiotensin II type 1 receptor antibody levels.

Supplementary Methods. Induction and maintenance immunosuppressive therapy protocols.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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