Circulating donor-specific anti-HLA antibodies are a major factor in premature and accelerated allograft fibrosis

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Addressing the causes of kidney allograft-accelerated aging is an important challenge for improving long-term transplant outcomes. Here we investigated the role of circulating donor-specific anti-HLA antibodies (HLA-DSAs) in the development and the progression of kidney allograft fibrosis with inclusion of traditional risk factors for allograft fibrosis. We prospectively enrolled 1539 consecutive kidney recipients transplanted in two centers and assessed interstitial fibrosis and tubular atrophy (IF/TA) in biopsies performed at one year post-transplantation. The HLA-DSAs and all traditional determinants of IF/TA were recorded at transplantation and within the first year post-transplantation, including histological diagnoses in 2260 "for cause" biopsies. This identified 498 (32%) patients with severe IF/TA (Banff IF/TA grade 2 or more). HLA-DSAs were significantly associated with severe IF/TA (adjusted odds ratio, 1.53; 95% confidence interval 1.16–2.01) after including 37 determinants. HLA-DSAs remained significantly associated with severe IF/TA in patients without antibody-mediated rejection (adjusted odds ratio 1.54; 1.11–2.14). HLA-DSAs were the primary contributor, being involved in 11% of cases, while T cell–mediated rejection, calcineurin-inhibitor toxicity, acute tubular necrosis, pyelonephritis, and BK virus–associated nephropathy were involved in 9%, 8%, 6%, 5%, and 4% of cases, respectively. One hundred fifty-four patients with HLA-DSA–associated severe IF/TA showed significantly increased microvascular inflammation, transplant glomerulopathy, C4d deposition in capillaries, and decreased allograft survival compared to 344 patients with severe IF/TA without HLA-DSAs. Three hundred seventy-eight patients with post-transplant HLA-DSAs exhibited significantly accelerated progression of IF/TA compared to 1161 patients without HLA-DSAs in the biopsies performed at one year post-transplant and beyond. Thus, circulating HLA-DSAs are major determinants of premature and accelerated allograft fibrosis acting independently of traditional risk factors and antibody-mediated rejection.

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The development of organ fibrosis represents the common result of past or active (or both past and active) multiple conditions and leads to tissue injury and organ failure.[1] In the field of solid organ transplantation, fibrosis is recognized as a hallmark of failing allografts in kidney, liver, and pancreatic transplants.[2–4]

Many contributors to interstitial fibrosis in kidney allograft have been described in the literature,[5,6] including donor characteristics,[7–12] transplant parameters,[13–17] and post-transplantation recipient conditions, such as calcineurin inhibitor (CNI) toxicity,[6,18–21] infectious diseases,[22–26] hypertension,[27,28] and rejection.[5,29–32] Significant overlap among the numerous determinants of kidney allograft interstitial fibrosis has made the precise quantification of their respective attributable contributions challenging. Addressing the etiological heterogeneity of interstitial fibrosis and identifying phenotypes based on pathophysiology is critical for improving the longevity of existing organ allografts. The importance of precise disease phenotyping based on the involved pathogenic mechanisms for personalizing care and improving outcome has been demonstrated in many fields (cancer, cardiovascular disease, and immunity-related and infectious diseases).[33]

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The occurrence and progression of interstitial fibrosis in kidney is intimately linked to endothelial activation as evidenced by *in vitro* experiments and animal models.[34–39] In humans, the relationship between immunological endothelial activation and fibrogenesis has been demonstrated in native kidneys under numerous conditions, such as systemic sclerosis[40,41] and lupus erythematosus.[42–45] In kidney allograft, among the major causes of endothelial activation is the binding of circulating donor-specific antihuman leukocyte antigen donor-specific antibodies (anti-HLA-DSAs) to endothelial cells that contribute to the process of antibody-mediated allograft rejection.[46–49] Anti-HLA-DSAs are also considered strong determinants of long-term kidney allograft failure.[50–52] Nevertheless, chronic allograft glomerulopathy is the only histological feature of chronic allograft deterioration that has been linked to circulating anti-HLA-DSAs in the current international Banff classification.[53] To date, we have an incomplete understanding of the respective contributions of circulating anti-HLA-DSAs and traditional immune and nonimmune risk factors to interstitial fibrosis progression. In addition, the specific influence of these factors on the interstitial fibrosis phenotype remains unknown.

In view of the profound interactions among the multiple determinants of interstitial fibrosis in kidney allograft, we aimed (i) to assess the contribution of anti-HLA antibodies to premature severe interstitial fibrosis independently of immune and nonimmune risk factors and (ii) to address the specific allograft interstitial fibrosis phenotype associated with circulating anti-HLA-DSAs in a population-based study. Patients were assessed for all kidney allograft fibrosis risk factors that have been reported in the literature, as well as circulating anti-HLA antibodies, and underwent systematic kidney allograft biopsies to evaluate the severity and the progression of interstitial fibrosis and tubular atrophy (IF/TA).

RESULTS

Characteristics of the study population

We included 1539 patients who received kidney transplants between January 1, 2004, and December 31, 2010. Two distinct populations were identified according to the severity of IF/TA on the allograft biopsy as performed at 1 year posttransplantation: (i) kidney transplant recipients with minimal IF/TA (Banff IF/TA grade of 0 or 1; that is 0%–25% of cortical area; $n = 1041$, 67.6%) and (ii) kidney transplant recipients with severe IF/TA (Banff IF/TA grade of 2 or 3; that is >25% of cortical area; $n = 498$, 32.4%). The baseline characteristics of the study population are listed according to the severity of IF/TA at 1 year posttransplantation in Table 1.

Patients with severe IF/TA at 1 year were significantly older (50.4 ± 13.4 years old) than those with minimal IF/TA at 1 year (46.4 ± 13.2 years old; $P < 0.001$) and had received allografts from older donors (56.0 ± 15.3 vs. 47.7 ± 16.3 years old; $P < 0.001$) who were more often deceased due to a cerebrovascular event (53.6% vs. 38.8%; $P < 0.001$). Recipients with severe IF/TA at 1 year had a longer cold ischemia time (19.3 ± 9.3 vs. 15.7 ± 9.9 hours; $P < 0.001$) and were more likely to have circulating anti-HLA-DSAs at the time of transplantation (25.7% vs. 18.4%; $P = 0.001$) compared with those with minimal IF/TA at 1 year.

Patients with severe IF/TA at 1 year presented with lower estimated glomerular filtration rate (eGFR) (44.4 ± 16.2 ml/min per 1.73 m^2 vs. 56.5 ± 18.3 ml/min per 1.73 m^2; $P < 0.001$) at 1 year posttransplantation compared with patients with minimal IF/TA at 1 year.

Determinants of severe IF/TA at 1 year posttransplantation

Univariate analysis. Table 2 shows the univariate analysis of the associations between severe IF/TA at 1 year posttransplantation and (i) recipient baseline characteristics, (ii) donor characteristics, (iii) transplantation characteristics, (iv) allograft injuries occurring within the first-year posttransplantation, and (v) recipient clinical and immunological characteristics as assessed within the first-year posttransplantation among the 37 determinants of IF/TA that we considered.

Multivariate analysis. The independent determinants of severe IF/TA at 1 year posttransplantation were as follows (Table 3): donor and recipient sex (adjusted odds ratio [aOR], 1.87; 95% confidence interval [CI], 1.44–2.42; $P < 0.001$, and aOR, 0.73; 95% CI, 0.56–0.93; $P = 0.012$, respectively); donor age (aOR, 1.02; 95% CI, 1.01–1.03; $P < 0.0001$), donor type ($P < 0.001$), donor comorbidities including diabetes mellitus (aOR, 2.35; 95% CI, 1.29–4.26; $P = 0.005$) and hypertension (aOR, 1.40; 95% CI, 1.04–1.88; $P = 0.028$); delayed graft function (aOR, 1.54; 95% CI, 1.17–2.03; $P = 0.002$); allograft injuries occurring within the first year posttransplantation including T-cell–mediated rejection (TCMR) (aOR, 2.09; 95% CI, 1.47–2.97; $P < 0.001$), BK virus–associated nephropathy (BKVAN) (aOR, 2.84; 95% CI, 1.33–6.08; $P = 0.007$); and recipient characteristics assessed at 6 months posttransplantation: eGFR (aOR, 0.97; 95% CI, 0.97–0.98; $P < 0.001$), proteinuria (aOR, 1.16; 95% CI, 1.00–1.34; $P = 0.046$), and body mass index (aOR, 0.95; 95% CI, 0.92–0.98; $P < 0.001$). The presence of circulating anti-HLA-DSAs was independently associated with severe IF/TA at 1 year posttransplantation (adjusted aOR, 1.53; 95% CI, 1.16–2.01; $P = 0.002$). HLA class II anti-HLA-DSAs were associated with higher levels of IF/TA at 1 year compared with HLA class I anti-HLA-DSAs (mean IF/TA Banff grade, 1.39 ± 1.11 vs. 1.13 ± 1.08, respectively; $P = 0.036$).

Sensitivity analysis based on preimplantatory biopsies. Among the 1539 kidney recipients included in the study, 913 (59.3%) had undergone preimplantatory histologic evaluation performed at day 0. On these biopsies, IF/TA Banff grade distribution was as follows: 726 (79.5%) IF/TA0, 145 (15.9%) IF/TA1, 36 (3.9%) IF/TA2, and 6 (0.7%) IF/TA3. The histologic characteristics of the allograft biopsies performed at day 0 are presented in Supplementary Table S1. In patients with day-0 biopsy, the presence of posttransplant anti-HLA-DSAs remained associated with severe IF/TA at 1 year independently of IF/TA severity at day 0 (OR, 1.71; 95% CI, 1.25–2.34; $P = 0.001$).