Angiotensin II Type 1 receptor antibodies are associated with inflammatory cytokines and poor clinical outcomes in pediatric kidney transplantation

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Angiotensin II type 1 receptor (AT1R) antibody has been linked to poor allograft outcomes in adult kidney transplantation. However, its clinical consequences in children are unknown. To study this, we examined the relationship of AT1R antibody with clinical outcomes, biopsy findings, inflammatory cytokines, and HLA donor-specific antibodies (DSA) in a cohort of pediatric renal transplant recipients. Sixty-five patients were longitudinally monitored for AT1R antibody, HLA DSA, IL-8, TNF-α, IL-1β, IFN-γ, IL-17, and IL-6, renal dysfunction, hypertension, rejection, and allograft loss during the first two years post transplantation. AT1R antibody was positive in 38 of the 65 of children but was not associated with HLA DSA. AT1R antibody was associated with renal allograft loss (odds ratio of 13.1 [95% confidence interval 1.48–1728]), the presence of glomerulitis or arteritis, and significantly higher TNF-α, IL-1β, and IL-8 levels, but not rejection or hypertension. AT1R antibody was associated with significantly greater declines in eGFR in patients both with and without rejection. Furthermore, in patients without rejection, AT1R antibody was a significant risk factor for worsening eGFR over the two-year follow-up period. Thus, AT1R antibody is associated with vascular inflammation in the allograft, progressive decline in eGFR, and allograft loss. AT1R antibody and inflammatory cytokines may identify those at risk for renal vascular inflammation and lead to early biopsy and intervention in pediatric kidney transplantation.

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Antibody-mediated rejection (AMR) remains a significant barrier to successful long-term outcomes in kidney transplantation.[1–3] The role of alloantibody responses against human leukocyte antigens (HLAs) in mediating AMR has been a primary focus in transplantation.[2,4,5] However, non-HLA autoantibodies have gained importance for their involvement in AMR.[6–8] Moreover, the interplay between alloantibody and autoantibody responses is becoming important to our understanding and management of AMR. Antibodies to various non-HLA targets,[6,7] such as major histocompatibility complex class I–related chain A,[9–14] endothelin type A receptor,[15,16] perlecan,[17] collagen IV,[18] fibronectin,[18] and angiotensin II type 1 receptor antibody (AT_1R-Ab)[19–24] have been associated with poor allograft outcomes in renal transplantation. Evidence for routine testing, however, remains insufficient.[8]

AT_1R-Ab, in particular, is becoming recognized for its association with vascular injury and allograft failure in adult kidney transplant patients.[19–24] Dragun *et al.*[19] were the first to report the association of AT_1R-Ab with acute AMR, endarteritis, and severe hypertension in kidney transplant recipients. Since that time, AT_1R-Ab has also been linked to AMR in the absence of hypertension, allograft loss, acute cellular rejection, and decreased renal function in patients without this classic presentation,[21–25] suggesting multiple clinical phenotypes exist for AT_1R-Ab–mediated allograft injury.

AT_1R-Ab can directly injure endothelial and vascular smooth muscle cells, leading to elevated transcription factors associated with proinflammatory responses.[26] Therefore, peripheral blood markers of inflammation such as cytokines might enhance our understanding of the effects of AT_1R-Ab in clinical transplantation. Additionally, the relationship between allograft injury due solely to AT_1R-Ab versus that due to the combination of AT_1R-Ab and HLA donor-specific antibody (DSA) is unclear. Certain studies have found that the pathogenesis of AT_1R-Ab–induced injury is independent of HLA DSA,[24] whereas others have found that AT_1R-Ab and HLA DSA together portend inferior clinical outcomes.[22]

Recently, high AT_1R-Ab levels have been shown to be more common in pediatric than adult renal transplant recipients.[27,28] However, the clinical significance and impact of elevated AT_1R-Ab levels on allograft outcomes in pediatric

patients remain unclear. Therefore, we examined the relationship of AT_1R-Ab with clinical outcomes including allograft loss, biopsy findings, and renal function in a longitudinal cohort of pediatric renal transplant recipients. In addition, we investigated the association of AT_1R-Ab with HLA DSA and a panel of inflammatory cytokines.

RESULTS

Prevalence and clinical characteristics

The prevalence of AT_1R-Ab >17 U/ml in our pediatric cohort was 58% (38/65) at any time pre-transplantation to 2 years post-transplantation. The cutoff of 17 U/ml was initially chosen based on the literature.[20,29] Furthermore, we generated an area under the curve for AT_1R-Ab and clinical outcomes, which confirmed a cutoff of 17 U/ml was within 6% of the optimal threshold in our pediatric cohort (Supplementary Table S1). In AT_1R-Ab–positive patients, AT_1R-Ab was present before transplantation (i.e., preformed) in 39% (15/38), *de novo* after transplantation in 45% (17/38), and undetermined in 16% (6/38) due to the lack of pre-transplantation sera. Of the patients in whom *de novo* AT_1R-Ab developed, it developed in 59% (10/17) in the first 6 months, 35% (6/17) between 6 and 12 months, and 6% (1/17) between 12 and 24 months post-transplantation. Figure 1 shows the longitudinal comparison of AT_1R-Ab levels in AT_1R-Ab– positive (Figure 1a) versus AT_1R-Ab–negative (Figure 1b) patients during the first 2 years post-transplantation. Patients who were AT_1R-Ab positive at any time during the monitoring period generally remained positive throughout. All patients who were positive pre-transplantation continued to be AT_1R-Ab positive at some time post-transplantation.

There were no significant differences in demographic or baseline clinical characteristics between AT_1R-Ab–positive (defined as >17 U/ml at any time point) and AT_1R-Ab–negative patients (Table 1). Table 2 compares immunologic characteristics and therapy between the 2 groups. There were no differences in pre-transplantation sensitization risk factors including HLA mismatch, panel reactive antibody (PRA), and history of transplantation. There was an association between AT_1R-Ab and antithymocyte globulin (ATG) induction ($P = 0.037$). Four of 6 patients given ATG induction were sensitized with PRA Class I or II >30%, and 2 were given ATG as part of a rapid steroid withdrawal protocol. The development of HLA DSA was notably not a risk factor for AT_1R-Ab ($P > 0.99$). Additional immunomodulatory treatments beyond our standard immunosuppression protocol (Table 2) were given for delayed graft function, acute rejection, or disease recurrence. Overall, patients in the AT_1R-Ab–positive group received more total treatment days of augmented immunomodulation ($P = 0.010$) and plasmapheresis ($P = 0.002$). Using a mixed-effects longitudinal regression model, we found no temporal association between AT_1R-Ab and the development of HLA DSA, acute rejection, or immunomodulatory treatments including plasmapheresis (data not shown).

AT_1R-Ab and allograft loss

AT_1R-Ab–positive status within the first 2 years post-transplantation was associated with renal allograft loss ($P = 0.036$), but not rejection or hypertension (Figure 2). Seven patients experienced allograft loss: 1 between 0 and 6 months, 3 between 6 and 12 months, and 3 between 12 and 24 months. Five of 7 patients were AT_1R-Ab positive on the sample prior to allograft failure. The remaining 2 patients were AT_1R-Ab positive at the time of treatment-resistant acute cellular rejection episodes with vascular involvement and were treated with rituximab and/or bortezomib plus plasmapheresis. The AT_1R-Ab became negative after treatment; however, in these 2 patients progressive fibrosis and allograft failure subsequently developed.

We conducted univariate and multivariable analysis to further assess risk factors for allograft loss (Supplementary Table S2). We limited the number of variables in our model given the small number of events and excluded potential intermediate outcomes. On univariate analysis, AT_1R-Ab–positive status (odds ratio, 13.1; 95% CI 1.48–1728.44; $P = 0.036$), deceased donor transplant ($P = 0.038$), mean HLA mismatch ($P = 0.005$), and physician-assessed nonadherence ($P = 0.008$) were associated with renal allograft loss (Supplementary Table S2). As significant collinearity existed between donor type and HLA mismatch, only HLA mismatch was included in the final model (see Statistical Methods). AT_1R-Ab–positive status remained associated with allograft loss (odds ratio, 9.24; 95% confidence interval 0.51–168.38; $P = 0.061$) after accounting for mean HLA mismatch and physician-assessed nonadherence in the multivariable model (Supplementary Table S2).

AT_1R-Ab and biopsy findings

AT_1R-Ab was not associated with acute cellular rejection, C4d negative AMR, or C4d positive–AMR (Supplementary Table S3). AT_1R-Ab was not associated with increased acute interstitial or tubular inflammation scores. A combination score reflecting the presence of vascular inflammation represented by either glomerulitis or arteritis was statistically significant ($P = 0.037$). AT_1R-Ab was not associated with acute peritubular capillaritis or other combination acute vascular inflammation scores. AT_1R-Ab was not associated with chronic change scores or degree of interstitial fibrosis and tubular atrophy (Supplementary Table S3).

AT_1R-Ab and renal function

Patients with AT_1R-Ab demonstrated greater decreases in eGFR over the first 2 years post-transplantation compared with those without AT_1R-Ab ($P = 0.013$) (Figure 3a). Because AT_1R-Ab may exert direct effects on the allograft endothelium outside the context of biopsy-proven acute rejection, we investigated AT_1R-Ab as a risk factor for worsening renal function in patients both with and without rejection. In both groups, patients with AT_1R-Ab had a greater median decrease in eGFR than those without AT_1R-Ab ($P = 0.003$) (Figure 3b). Furthermore, we longitudinally analyzed eGFR in