HHS Public Access

Author manuscript

Pediatr Transplant. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Pediatr Transplant. 2015 August ; 19(5): 499–503. doi:10.1111/petr.12531.

De Novo Development of Antibodies to Kidney-Associated Self-Antigens Angiotensin II Receptor Type I, Collagen IV, and Fibronectin Occurs at Early Time Points after Kidney Transplantation in Children

Laura E Hesemann, MD[1], **Vijay Subramanian, MD**[2], **Thalachallour Mohanakumar, PhD**[2], and **Vikas R Dharnidharka, MD, MPH**[1]

[1]Department of Pediatrics, Division of Pediatric Nephrology, Washington University in St. Louis, St. Louis, MO, United States

[2]Department of Surgery, Pathology and Immunology, Washington University in St. Louis, St. Louis, MO, United States

Abstract

Background—Chronic rejection is the leading cause of graft loss following pediatric kidney transplantation. Our laboratory and others have demonstrated an association between development of antibodies to self-antigens and chronic rejection following adult lung and heart transplantation. The goal of this study was to determine whether antibodies to kidney-associated self-antigens develop following pediatric renal transplantation.

Objective—We investigated post-transplant development of antibodies to kidney-associated self-antigens Angiotensin II Receptor Type I, Fibronectin, and Collagen IV in a pediatric cohort.

Design/Methods—Using ELISA, we measured antibodies to kidney-associated self-antigens in serum. Our cohort included 29 subjects with samples collected pre-transplant and for 12 months post-transplant.

Results—No samples had antibodies to kidney-associated self-antigen pre-transplant. In contrast, 50% (10/20) of subjects developed antibodies to one or more kidney-associated self-antigen post-transplantation. The median time to antibody appearance and duration of persistence were 103 and 61 days, respectively. Development of antibodies did not correlate with graft function.

Conclusions—Half of subjects developed antibodies to kidney-associated self-antigens Angiotensin II Receptor Type I, Fibronectin, or Collagen IV in the first year after kidney transplantation - a higher rate of early antibody development than expected. In this small study, antibodies did not correlate with worse clinical outcomes.

Correspondence: Vikas R. Dharnidharka, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8116, St. Louis, Missouri 63110, vikasd@wustl.edu.

Conflict of Interest Statement

The authors of this manuscript have no relevant conflicts of interest to disclose as described by *Pediatric Transplantation*.

Parts of this work were presented in abstract form at Pediatric Academic Societies Annual Meeting, Vancouver, Canada, 2014.

Keywords

renal transplantation; chronic rejection; autoantibody

Introduction

In the last two decades, the use of newer more potent immunosuppressive agents has resulted in a dramatic decrease in the rate of early acute rejection, yet there has been no significant improvement in long-term allograft survival [1]. One of the major reasons for the lack of improvement in long-term allograft survival is chronic rejection, an irreversible injury to the graft that results in fibrosis and a decrease in function. This lack of improvement is an important health barrier for many patients as organ transplantation is the treatment of choice for end-stage disease of the heart, lungs, and kidneys in children, as well as in many adults. The impact of long term graft loss is even more significant in children as their long-term survival and quality of life will often require repeat transplantation, possibly multiple times during their lives.

There have been several mechanisms proposed to contribute to chronic rejection, including immunological and infectious processes. Viral infections are associated with chronic allograft rejection in various transplanted organs [2]. Early work in heart transplantation linked cytomegalovirus (CMV) infection to allograft vasculopathy, a classic phenotype of chronic rejection [3, 4]. A previous study in children found that even subclinical viremia was associated with a higher incidence of chronic rejection in renal allografts at 3 years after transplantation [5]. Other studies have linked acute rejection and allograft dysfunction to infection with several other viruses [6]. Subclinical infection is also associated with inferior graft function at 2 or 3 years post-transplant [7]. Bacterial infections have also demonstrated similar effects.

Immune responses directed towards tissue-associated self-antigens have also been demonstrated to have a significant role in the development of chronic rejection. Our group has previously shown a strong association between the development of antibodies to lung-associated self-antigens Kα1 Tubulin and Collagen V and the development of chronic rejection following human lung transplantation in an adult cohort. We have also demonstrated increased immune responses to Fibronectin (Fn), and Collagen IV (Col IV) following kidney transplantation in adults with biopsy-proven transplant glomerulopathy [8, 9]. Others have shown correlation between antibodies to Angiotensin II Receptor, Type I (ATR1) and allograft loss [10].

Although the exact mechanisms by which these autoantibodies develop or contribute to rejection is unclear, it is proposed that they may develop when cryptic self-antigens previously hidden from the immune system are exposed due to tissue injury such as may occur during infection.

The presence of antibodies to kidney-associated self-antigens has not been previously-studied in children. Because of the high rate of both clinically-apparent and subclinical infections in children, we hypothesized that pediatric kidney transplant recipients would

develop circulating antibodies to kidney-associated self-antigens ATR1, Col IV, and Fn, and that this may have an impact in the development of chronic rejection.

Methods

We performed a retrospective cohort study using samples obtained from 2010 to 2011 from pediatric kidney transplant recipients from Shands Children's Hospital, Gainesville, Florida that had been stored for future research use with IRB approval for other immunological testing [11]. This study was also approved by the Human Research Protection Office at Washington University in St. Louis, IRB Approval # 201210079. Demographic information of the entire study cohort is shown in Table 1. The standard immunosuppressive protocol for these patients consisted of induction with rabbit antithymocyte globulin given over 3-5 days followed by maintenance tacrolimus and mycophenolate. Steroids were reserved for specific situations.

Detection of Abs to self-antigen ATR1

Serum samples were stored at -80°C and were thawed at room temperature on the day the assays were performed. We tested samples for the presence of antibodies to ATR1 using a commercially-available enzyme-linked immunosorbent assay (ELISA) (Cusabio, Wuhan, China). Samples were diluted to a concentration of 1:25 using the sample diluent provided. Immunosorbence was measured at 450nm, and original concentration calculated using a standard curve obtained through serial dilution of manufacturer-provided standard of known concentration. A positive result was defined as a value greater than 2 standard deviations above the mean concentration of antibody measured in sera of a control group of healthy adult volunteers.

Detection of Abs to self-antigens Col IV and Fn

Serum samples were stored at −80°C and were thawed at room temperature on the day the assays were performed. We tested samples for the presence of antibodies to Col IV and Fn using an ELISA developed in our laboratory using techniques previously-described [9]. Commercially-available anti-Fn and anti-Col IV were used as positive controls (Santa Cruz Biotechnology, Santa Cruz, CA). Antibody concentrations were calculated by normalizing optical density of samples to those of known standards. Positivity was defined as previously-stated using a control group of healthy adult volunteers.

Correlation of Abs to self-antigens and post-transplantation events and outcomes

The original study protocol for which the samples were collected included monthly PCR testing for CMV, Epstein-Barr virus (EBV), and BK virus (BK). Other infection events such as urinary tract infection, bacteremia, and sepsis were recorded separately. Using chi-square test with two-sided p-value of 0.05, we tested for association between infection event and presence of any level of antibody to any self-antigen. We used the Mann-Whitney U test to evaluate the association between presence of antibodies and graft function calculated as estimated glomerular filtration rate (eGFR) at 1 year. Estimated GFR was calculated in all subjects using the modified Schwartz formula[12].

Results

Pre-existing antibodies to kidney-associated self-antigens ATIR, Fn, and COL IV in pre-transplant sera of patients waiting for pediatric kidney transplantation

To detect the pre-transplant prevalence of antibodies to kidney-associated self-antigens, we analyzed 28 pre-transplant samples for Ab to ATR1 and 20 for Ab to Fn and Col IV. No sample had significant levels of any of the antibodies.

Development of antibodies to kidney-associated self-antigens following renal transplantation in children

In order to determine the *de novo* development of Abs post-transplant, we analyzed serially-obtained post kidney transplant samples in children. We tested 144 post-transplant samples from 20 subjects for Abs to ATR1, 81 samples for Abs to Fn, and 83 samples for Abs to Fn and Col IV. Variation in the number of samples tested for each antibody was due to limited quantity of serum available as these samples were aliquots remaining from a previous study. As shown in Table 2, 25 samples from 8 different subjects were positive for Abs to ATR1. Eight samples from 3 different subjects were positive for Abs to Fn. One subject had Abs to both ATR1 and Fn. No subjects had Abs to Col IV. The earliest development of any Ab was 16 days post-transplantation with median time to Ab development 103 days (range 16-170). It is important to note that all Abs to kidney-associated self-antigens detected were due to *de novo* development of immune responses to these self-antigens.

Analysis of development of antibodies to kidney-associated self-antigens and its correlation with graft function

The mean eGFR at 1 year post-transplant in those subjects who developed antibodies to kidney-associated self-antigens was 80.3 ml/min/1.73m^2 +/− 26 compared to 56.7 ml/min/1.73m^2 +/− 30.5 in those without antibodies. This difference was not statistically significant (p=0.19).

Analysis of development of antibodies to kidney-associated self-antigens and its correlation with post-transplant events in children

To determine if the development of antibodies to kidney-associated self-antigens correlated with post-transplant infections, we evaluated the results of the autoantibody assays relative to the recorded history of infection events (Table 3). A total of 19 infection events (12 viral, 7 bacterial) occurred in 13 subjects. Seven (37%) of these episodes had a rise in autoantibody titer concomitantly or at the next sampling point. The rise was seen in AT1R Ab after 4 events in 3 subjects. A rise in Fn Ab occurred after 4 events in 3 subjects. Two events in two subjects had rises in both AT1R Ab and Fn Ab concomitantly. However, Ab rise without concomitant or preceding infection was also seen frequently. AT1R Ab rise without a concomitant or preceding infection was seen 9 different times in 5 subjects. The results did not reveal any specific pattern.

We evaluated the association between development of antibodies to kidney-associated self-antigens and acute rejection and to the presence of donor specific antibodies (DSA) (Table 3). Seven of 20 subjects, including 2 of the 10 patients with Ab development, experienced

acute rejection during the study period. Two of the 10 patients with Ab development also developed DSA during the study period. Neither association was statistically significant.

Discussion

De novo development of antibodies to self-antigens has been shown to correlate with chronic graft dysfunction in adult kidney transplantation [9]. In this study we demonstrate that pediatric transplant recipients also develop *de novo* circulating antibodies to kidney-associated self-antigens ATR1, Fn, and Col IV. This is not surprising given that the theorized mechanism for the development of these antibodies involves exposure of previously-hidden antigens following tissue damage. Because the same processes which lead to tissue damage in adult transplant recipients are likely to occur in pediatric transplant recipients, it follows that the outcome should be similar. The overall rate of *de novo* development of antibodies to kidney-associated self-antigens, i.e. positive for any antibody, was similar to that seen in previous studies of adult cohorts of solid organ transplant patients at later time points who had a known diagnosis of chronic rejection. The majority of positivity in our study was for anti-ATR1 Ab with relatively lower development of antibodies to Fn or Col IV. The reason for this imbalance is unknown. To our knowledge, this is the first report of the *de novo* development of antibodies to kidney-associated self-antigens following pediatric kidney transplantation.

The mechanism for the development of antibodies to tissue-restricted self-antigens is not well-understood. We hypothesize that pre-transplant inflammation due to infection may expose cryptic self-antigens to the immune system. However, our preliminary analysis did not demonstrate a significant correlation between infection and development of antibodies. This is likely due to the small sample size of our pilot study as well as a lack of adequate number of serial samples. It may also represent a difference in immune responses in children versus adults, such as a different immune response to primary infection vs reactivation of prior viral infection as is often seen in adults.

Although we hypothesized that tissue injury due to underlying disease processes may expose antigens capable of inducing an immune response prior to transplantation, we did not find antibodies in any of the pre-transplant samples. In adult lung transplantation, it has been suggested that pre-transplant antibodies to lung-associated self-antigens Collagen V and K alpha 1 tubulin are associated with early graft dysfunction and chronic rejection [13]. Therefore, it is likely that the presence of these preexisting antibodies may be a risk factor for development of rejection though we were unable to demonstrate similar findings during this study, possibly due to small sample size.

There are several limitations of our study. The results are limited by lack of correlation to clinical outcomes such as the development of chronic rejection. It is possible that these samples were collected too early in the post-transplant period for the development of chronic rejection. Surveillance biopsies to establish the diagnosis of chronic rejection were not part of routine post-transplant monitoring at the center where the samples were originally collected. Additionally, we did not have data on several other aspects related to the subjects'

peri-operative and post-transplant courses including ischemia times and the individual immunosuppression received by each subject.

In summary, this study provides evidence for the first time for the *de novo* development of antibodies following pediatric kidney transplantation. However, there are several important unanswered questions including but not limited to their role in development of chronic rejection in children, mechanisms by which these antibodies develop and whether they correlate with disease severity or can predict the development of chronic rejection. Further studies are required to assess whether the prevention of antibody development may lead to prevention of chronic rejection and improve long-term graft outcomes, and whether any targeted treatment towards preventing development of antibodies to self-antigens would improve long-term graft outcomes.

Acknowledgments

This study was supported in part by a Pilot Project Award from the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences. LEH was supported through the Mentored Training Program in Clinical Investigation through the same grant. TM is supported by BJC foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

1. Meier-Kriesche HU, et al. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant. 2004; 4(3):378–383. [PubMed: 14961990]
2. Kotton CN, Fishman JA. Viral infection in the renal transplant recipient. J Am Soc Nephrol. 2005; 16(6):1758–1774. [PubMed: 15829710]
3. Graham JA, et al. Viral infection induces de novo lesions of coronary allograft vasculopathy through a natural killer cell-dependent pathway. Am J Transplant. 2009; 9(11):2479–2484. [PubMed: 19843029]
4. Grattan MT, et al. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA. 1989; 261(24):3561–3566. [PubMed: 2542633]
5. Smith JM, et al. Subclinical viremia increases risk for chronic allograft injury in pediatric renal transplantation. J Am Soc Nephrol. 2010; 21(9):1579–1586. [PubMed: 20616168]
6. Schowengerdt KO, et al. Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection: diagnosis using the polymerase chain reaction. Circulation. 1997; 96(10): 3549–3554. [PubMed: 9396454]
7. Li L, et al. Subclinical cytomegalovirus and Epstein-Barr virus viremia are associated with adverse outcomes in pediatric renal transplantation. Pediatr Transplant. 2007; 11(2):187–195. [PubMed: 17300499]
8. Saini D, et al. Alloimmunity-induced autoimmunity as a potential mechanism in the pathogenesis of chronic rejection of human lung allografts. J Heart Lung Transplant. 2011; 30(6):624–631. [PubMed: 21414808]
9. Angaswamy N, et al. Immune responses to collagen-IV and fibronectin in renal transplant recipients with transplant glomerulopathy. Am J Transplant. 2014; 14(3):685–693. [PubMed: 24410875]
10. Taniguchi M, et al. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. Am J Transplant. 2013; 13(10):2577–2589. [PubMed: 23941128]
11. Dharnidharka VR, et al. Verification of association of elevated serum IDO enzyme activity with acute rejection and low CD4-ATP levels with infection. Transplantation. 2013; 96(6):567–572. [PubMed: 23823655]

12. Schwartz GJ, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009; 20(3):629–637. [PubMed: 19158356]

13. Bharat A, et al. Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection. Ann Thorac Surg. 2010; 90(4):1094–1101. [PubMed: 20868794]

Table 1

Demographics

	Pre-Transplant Cohort n=28	**Post-Transplant Cohort n=20**
Age, yrs – median	12.4 (1.4–18.6)	12.3 (1.4–17.3)
Male	17 (61%)	14 (70%)
Race		
Caucasian	16 (57%)	15 (75%)
African-American	9 (32%)	5 (25%)
Hispanic	3 (10%)	0
Donor		
Living	6 (21%)	4 (20%)
Deceased	22 (79%)	16 (80%)
Repeat transplant	3 (10%)	1 (5%)
Prior Dialysis		
hemodialysis	16 (57%)	10 (50%)
Peritoneal dialysis	10 (36%)	2 (10%)
none	2 (7%)	8 (40%)
Cause of ESRD		
Obstruction	7 (25%)	8 (40%)
SLE	5 (18%)	2 (10%)
Dysplasia	6 (21%)	4 (20%)
FSGS	4 (14%)	1 (5%)
Other*	6 (21%)	5 (25%)

*Other = nemaline rod myopathy, autosomal recessive polycystic kidney disease, calcineurin inhibitor toxicity, unknown

Table 2

de novo Development of Kidney-Associated Self-Antigens

	Samples Positive	Subjects Positive
Angiotensin Receptor, Type I	25 (17%)	8 (40%)*
Fibronectin	8 (10%)	3 (15%)*
Collagen IV	0	0

* One subject developed antibodies to both ATR1 and Fn (see Table 3).

Table 3

Subjects with Antibodies to Kidney-Associated Self-Antigens

Subject	Abs	Abs on Multiple Occasions*	Acute Rejection Ever	Infections	DSA Ever Present Post-Transplant
11	ATR1	+	+	Bacterial	+
16	ATR1			Viral	
17	ATR1	+		Bacterial	
18	ATR1	+		Viral	
19	ATR1	+			
21	ATR1	+			
23	ATR1	+			
24	Fn			Viral	
27	ATR1, Fn	+	+	Bacterial	
28	Fn	+		Bacterial, Viral	+

* Multiple Occasions defined as separated by at least 60 days

Abbreviations: Abs = antibodies; DSA = Donor Specific Antibodies; ATR1 = Angiotensin II Receptor, Type I; Fn = Fibronectin