Research Article
Volume 11:3, 2021

Journal of
Transplantation Technologies & Research

ISSN: 2161-0991 Open Access

Pre-treatment Modulation of Renal Allograft Chemokine: Glycosaminoglycan Pathways Reduces Transplant Rejection

Michelle Burgin[1], Roxana Beladi[1,2], Kyle Varkoly[1,2], Jordan R. Yaron[1], Liqiang Zhang[1], Steve T. Yeh[3], Jacqueline Kilbourne[1], Dara Wakefield[4], William Clapp[4], Alexandra R. Lucas[1]*

[1]*Biodesign Institute, Arizona State Univ, Tempe, AZ*

[2]*Kansas City Medical University, Kansas City, MO*

[3]*IONIS Pharmaceuticals, Inc Carlsbad, CA*

[4]*Department of Pathology, University of Florida, Gainesville, FL*

Abstract

With over a hundred thousand transplants needed every year and limited availability leading to the death of 20 patients each day, long term viability of solid organ transplants is imperative. Early allograft transplant rejection is well controlled by immunosuppression of T cells, but late organ loss due to allograft vascular (AV) disease and chronic immune damage are unmet medical needs. Chronic AV disease and ongoing immune damage are leading causes of late transplant organ loss. In prior work, we demonstrated that implant of an allograft kidney with a conditional deficiency of the N-deacetylase N-sulfotransferase 1 (Ndst1-/-) enzyme in the endothelium and myeloid precursors (C57Bl/6 background) led to a significant decrease in early rejection after implant into wild type BALB/c mice with normal Ndst1 expression.

Introduction

With over a hundred thousand transplants needed every year and limited availability leading to the death of 20 patients each day, long term viability of solid organ transplants is imperative. Early allograft transplant rejection is well controlled by immunosuppression of T cells, but late organ loss due to allograft vascular (AV) disease and chronic immune damage are unmet medical needs. Chronic AV disease and ongoing immune damage are leading causes of late transplant organ loss. In prior work, we demonstrated that implant of an allograft kidney with a conditional deficiency of the N-deacetylase N-sulfotransferase 1 ($Ndst1^{-/-}$) enzyme in the endothelium and myeloid precursors (C57Bl/6 background) led to a significant decrease in early rejection after implant into wild type BALB/c mice with normal Ndst1 expression [1-3].

This finding suggested that pre-transplant modification of the endothelial polysaccharide surface layer (EPSL) and glycosaminoglycans (GAGs) in donor organs may be beneficial in reducing early and late AV disease and subsequent rejection. The glycocalyx has a central role in the immune response as it serves as a scaffold for new cell growth and chemokine-directed immune cell recruitment [4]. Prior literature has also demonstrated marked improvement in both early and late rejection after treatment with a chemokine modulating protein, M-T7 [5,6]. M-T7 is a 37KDa glycosylated protein that significantly reduces intimal hyperplasia following vascular injury. M-T7 blocks binding of C, CC, and CXC chemokine classes to glycosaminoglycans, interfering with development of chemokine gradients that direct immune cell migration. M-T7 given immediately after renal allograft transplants and for 10 days after transplant effectively reduces acute rejection and longer-term rejection when given with cyclosporine treatment. When given alone, M-T7 reduces aortic transplant inflammation and improves survival in mouse renal allografts [7,8].

The capacity for transplantation of donor allografts with Ndst1 deficiency to significantly reduce acute rejection suggests that modifying donor organs prior to transplantation can have profound effects on rejection and vasculopathy. We have examined pre-treatments designed to reduce normal GAG and chemokine interactions to reduce rejection. Donor renal allografts were treated with either antisense suppression of Ndst1 (ASO^{Ndst1}) or with M-T7 blockade of chemokine/ GAG interactions.

Methods

A series of subcutaneous and subcapsular renal allograft transplants were examined using C57Bl/6 renal sections (3 mm^2) engrafted into recipient BALB/c mice. Subcutaneous C57Bl/6 transplants were soaked in either saline, saline with antisense to Ndst1 (ASO^{Ndst1}), scrambled antisense (ASO^{Scr}), or M-T7. $Ndst1^{-/-}$ renal grafts were compared as controls. BALB/c to BALB/c implants provided isograft controls. ASO^{Ndst1} developed by IONIS was assessed in normal C57Bl/6 mice, and demonstrated a significant reduction in the expression of Ndst1 mRNA (Table 1).

As a second approach, subcapsular transplants of 3 mm^2 sections of C57Bl/6 kidneys were either pre-treated by soaking for one hour prior to implantation or by injecting donor C57Bl/6 mice daily for 7 days with M-T7, ASO^{Ndst1}, ASO^{Scr}or saline by intraperitoneal (IP) injection prior to harvest of the kidney for transplant. Up to 8 transplants per treatment group/ transplant approach were assessed (Table 1). Allograft implants were assessed at either 3 or 15 days post-transplantation. No additional immune suppressant treatments were given.

68 BALB/c mice had subcutaneous transplant with pretreatment immediately prior to implantation into the intracapsular subcutaneous space: 12 with $Ndst1^{-/-}$ allografts, 19 WT C57Bl/6 treated with saline, 7 with M-T7, 14 ASO^{Ndst1}, and 10scranbled ASO control (ASO^{Scr}). 46 BALB/c mice had subcapsular transplants: 8 with $Ndst1^{-/-}$ allografts, 14 C57Bl/6 grafts treated with saline, 9 with M-T7 and 11 ASO^{Ndst1}, and 4 ASO^{Scr}. 48 C57Bl/6 donor mice were pretreated 7 days before donor allograft harvest for subcapsular transplant: 12 treated with saline, 12 with M-T7, 12 ASO^{Ndst1}, and 12 ASO^{Scr}.

***Address for Correspondence:** Alexandra R. Lucas, Biodesign Institute, Arizona State Univ, Tempe, AZ, Email: alexluc1@asu.edu*

Received 22 September 2020; **Accepted** 14 October 2020; **Published** 15 February 2021

Table 1. Table of transplant studies.

	Transplant Type	Numer of Days Follow up	Treatment	Number of Mice
Subcutaneous Soaked	BALB/c - BALB/c	15 Days	Saline	5
	NDST KO - BALB/c		Saline	6
	C57BL/6 - BALB/c		Saline	6
	C57BL/6 - BALB/c		MT7	3
	C57BL/6 - BALB/c		Scramble ASO	6
	C57BL/6 - BALB/c		NDST ASO	6
	C57BL/6 - BALB/c		CD40 ASO	6
	BALB/c - BALB/c	3 Days	Saline	6
	NDST KO - BALB/c		Saline	6
	C57BL/6 - BALB/c		Saline	2
	C57BL/6 - BALB/c		MT7	4
	C57BL/6 - BALB/c		Scramble ASO	4
	C57BL/6 - BALB/c		NDST ASO	8
Subcapsular Soaked	C57BL/6 - BALB/c	15 Days	Saline	10
	NDST KO - BALB/c		Saline	4
	C57BL/6 - BALB/c		MT7	5
	C57BL/6 - BALB/c		Scramble ASO	4
	C57BL/6 - BALB/c		NDST ASO	7
	C57BL/6 - BALB/c	3 Days	Saline	4
	NDST KO - BALB/c		Saline	4
	C57BL/6 - BALB/c		MT7	4
	C57BL/6 - BALB/c		Scramble ASO	0
	C57BL/6 - BALB/c		NDST ASO	4
Subcapsular Pretreated (7 Days Prior to Transplantation)	C57BL/6 - BALB/c	15 Days	Saline	6
	C57BL/6 - BALB/c		MT7	6
	C57BL/6 - BALB/c		Scramble ASO	6
	C57BL/6 - BALB/c		NDST ASO	6
	C57BL/6 - BALB/c	3 Days	Saline	6
	C57BL/6 - BALB/c		MT7	6
	C57BL/6 - BALB/c		Scramble ASO	6
	C57BL/6 - BALB/c		NDST ASO	6

All graft treatment solutions included oxygenated DMEM as a base to provide an environment best suited for graft survival. All renal transplants and animal studies conform to national and international guidelines for animal care and were approved by the local IACUC committee (BiodesignInst, ASU). Transplant surgeries were performed under general anesthesia.

Results

Subcutaneous kidney transplant

Ndst1$^{-/-}$ donor organs with conditional Ndst1 deficiency in endothelial cells and myeloid precursors in and the M-T7 soaked wild-type C57Bl/6 mice had reduced inflammation. ASO^{Ndst1} increased inflammation in this model (Figure 1). However, with subcutaneous implant there is marked ischemia due to the limited subcutaneous blood supply. In subsequent studies sub capsular renal allograft implants were examined with and without ASO and M-T7 treatment (Figure 1).

Sub capsular Kidney to Kidney transplantation with pretreatment at the time of transplant

M-T7 and ASO^{Ndst1} pre-treatment soaking for one hour in oxygenated DEM reduced inflammation. Effects of pre-treatment with ASO or M-T7 on selective change in F4/80 macrophage and CD3+ T cell invasion as well as subpopulations of M1, M2, Th1, Th2, T17 and Treg are in progress (Figure 2).

Sub capsular Kidney to Kidney transplantation with 7 days pre-treatment of the donor

MT-7 and ASO^{Ndst1} pre-treatments of donor C57BL/6 mice for 7 days prior to allograft harvest and transplantation reduces inflammation. Pretreatment with ASO^{Ndst1} reduced scar formation. The C4d rejection marker was reduced after M-T7 pretreatment significantly and after ASO^{Ndst1} pretreatment when compared to saline or ASO^{Scr}pretreatment. T-cell and macrophage staining is still in progress.

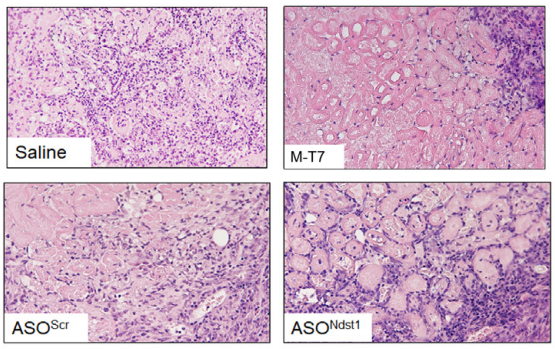

Figure 1. H&E staining of Subcutaneous Transplants. Marked inflammatory cell invasion is seen in Saline treated allografts. MT-7 treated allografts have reduced inflammation while ASO^{Ndst1} treated grafts had increased inflammation.

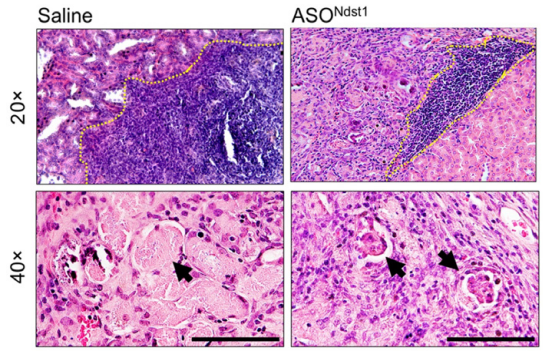

Figure 2. H&E staining of Subcapsular Transplants (15 days post-transplant) treated for one hour prior to subcapsular kidney to kidney transplants. 15 days post-transplant, Donor grafts pre-treated with ASO^{Ndst1} had decreased inflammation and preserved glomeruli (arrows) when compared to scarring in saline-treated allografts.

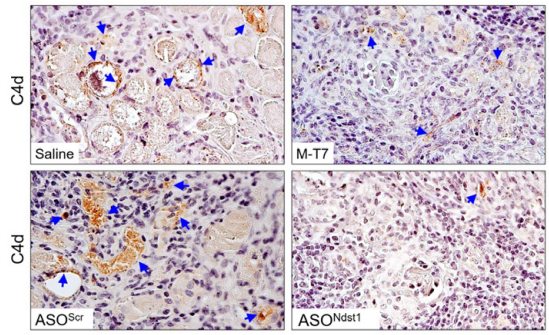

Figure 3. C4d staining of Subcapsular Transplants (15 days post-transplant). Pretreating donor C57BL/6 mice with M-T7 and ASO^{Ndst1} prior to organ harvest and engrafting decreases markers of antibody-mediated rejection (C4d, arrows) when compared to the saline and ASO^{Scr} treatment controls.

Correlations between chemokine and GAG composition as well as macrophage and T cell responses with rejection of renal allografts are in progress (Figure 3).

Conclusions

Pre-treatment of transplanted organs modifying GAG/chemokine interactions provides a new therapeutic approach to reducing acute and potentially chronic transplant immune damage and vasculopathy. There is a high demand and a limited supply of solid organs for transplantation. Pretreatments of donor organs has the potential to reduce rejection.

References

1. Health Resources and Services Administration. Organ Donation Statistics. US Government Information on Organ Donation and Transplantation. Accessed 2020 Sep 14th. https://www.organdonor.gov/statistics-stories/statistics.html
2. Weis M and von Scheidt W. "Cardiac allograft vasculopathy: a review." *Circulation* 96(1997):2069-2077.
3. Chen H, Ambadapadi S and Wakefield D. "Selective deletion of heparan sulfotransferase enzyme, Ndst1, in donor endothelial and myeloid precursor cells significantly decreases acute allograft rejection." *Sci Rep* 8(2018):13433.
4. Möckl L. "The emerging role of the mammalian glycocalyx in functional membrane organization and immune system regulation". *Front Cell Dev Biol*. (2020): 8, 253.
5. Bédard EL, Kim P and Jiang J. "Chemokine-binding viral protein M-T7 prevents chronic rejection in rat renal allografts." *Transplantation* 76(2003):249-252.
6. Dai E, Liu LY, and Wang H. "Inhibition of chemokine-glycosaminoglycan interactions in donor tissue reduces mouse allograft vasculopathy and transplant rejection". *PLoS One*. (2010): 5(5), e10510.
7. Liu L, Lalani A, and Dai E. "The viral anti-inflammatory chemokine-binding protein M-T7 reduces intimal hyperplasia after vascular injury." *J Clin Invest* 105(2000):1613-1621.
8. Liu L, Dai E, and Miller L. "Viral chemokine-binding proteins inhibit inflammatory responses and aortic allograft transplant vasculopathy in rat models." *Transplantation* 77(2004): 1652-1660.

How to cite this article: Michelle Burgin, Roxana Beladi, Kyle Varkoly, Jordan R. Yaron, Liqiang Zhang, Steve T. Yeh, et al. "Pre-treatment Modulation of Renal Allograft Chemokine: Glycosaminoglycan Pathways Reduces Transplant Rejection." *J Transplant Technol Res* 11 (2021): 180.