

Cystinosis Research Foundation Progress Report

Title: *Elucidating the role of aberrant macrophage activation in nephropathic cystinosis*

Grant number: 414010090101

Investigators: Daryl Okamura M.D.

Funding Period: September 1, 2013 to August 31, 2015

Progress Report: March 1, 2014 to August 31, 2014

OVERVIEW

The project is progressing and we have started to characterize the *Ctns*^{-/-} macrophage (MΦ) phenotype in response to kidney injury. As mentioned in our last progress report, we have been struggling with fungal contamination of our tissue culture incubator and supplies that did not resolve completely until June this year, partly due to shifting our attention to our in vivo studies. We have generated the chimeric mice and started functional experiments on the in vivo role of *Ctns*^{-/-} MΦ (Aim #2).

Aim #1: To determine the *Ctns*^{-/-} macrophage phenotype in response to cytokine activation and the mechanisms that lead to its altered behavior

Our initial hypothesis was that aberrant response to cytokine activation in *Ctns*^{-/-} MΦ resulted in more severe injury, however, our preliminary data below suggests that they attenuate injury. It is not clear if this is due to other factors such as proliferation/apoptosis or chemotaxis. Therefore, we will further characterize the in vivo data to help focus our in vitro investigations on mechanism.

AIM #2. To investigate the functional impact of the *Ctns*^{-/-} macrophage phenotype on regeneration and fibrosis after renal injury.

We generated *Ctns* ko/wt chimeric mice through bone marrow transplantation. Wild-type mice were irradiated with 900 rads and injected with bone marrow cells from *Ctns*^{-/-} mice retroorbitally. After 8-10 weeks to allow complete engraftment of the

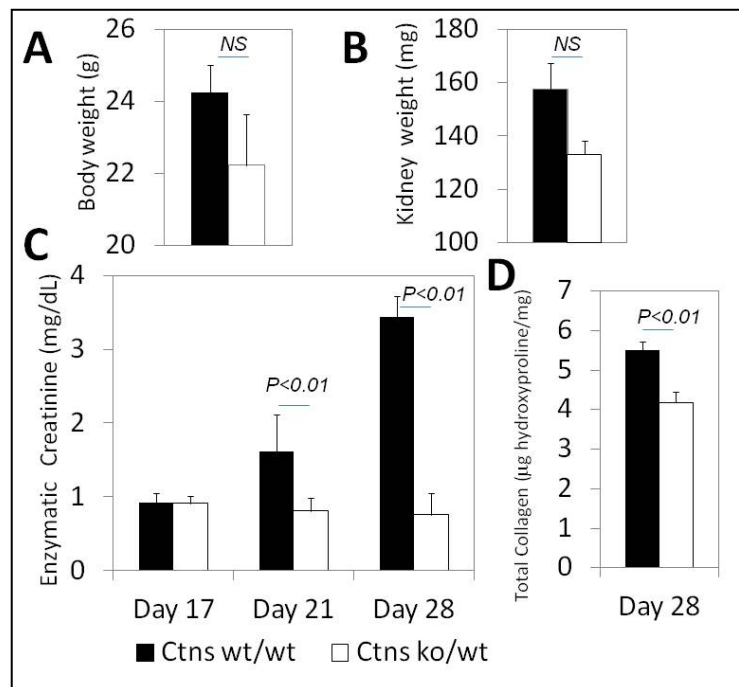


Figure 1. *Ctns* deficient macrophages attenuate the fibrotic response after ischemia reperfusion injury. (A,B) Body weight and kidney weight at day 28; (C) Kidney function was analyzed by enzymatic creatinine assay at day 17-28 after IR injury; (D) Total collagen was analyzed by measuring hydroxyproline content.

monocyte/macrophage population, Ctns ko/wt mice and Ctns wt/wt chimeric controls underwent unilateral ischemia reperfusion (IR) injury. After 14 days, both groups underwent contralateral nephrectomy to investigate kidney function of the IR kidney. Each mouse underwent blood draws at days 17, 21, and 28 after IR injury. Mice were sacrificed at day 28 and kidneys analyzed for fibrosis severity (n=5-8/group).

There was no difference in both body weight and kidney weight at time of sacrifice in Ctns ko/wt mice compared to chimeric controls (Fig 1A, B). Although there was no difference in creatinine at day 17 (3 days after nephrectomy) between Ctns ko/wt and chimeric controls, there was a continued progression and deterioration of kidney function in the chimeric control mice but not in the Ctns ko/wt mice (Fig 1C,D). There was approximately a 450 percent increase in creatinine between Ctns ko/wt and chimeric control mice at day 28. Analysis at fibrosis severity at time of sacrifice on day 28, confirmed our functional studies with a reduction in total collagen in Ctns ko/wt mice compared to chimeric controls. However, there was only a 24 percent reduction in total collagen compared to the much larger decrease in serum creatinine. Further characterization of the injury and histologic confirmation with picosirius red staining will enable us to further elucidate this discrepancy.

We also recently completed bone marrow transplantation using the DsRed Ctns^{-/-} mice from Dr. Cherqui that will allow us to follow the Ctns^{-/-} MΦ population in response to IR injury and during the fibrotic response.



Grantor Agency: Cystinosis Research Foundation

Total Award: \$256,208.00

Principal Investigator: Daryl Okamura

Co. Principal Investigator: _____

Research Fellow: _____

Effective Date of Grant: 9/1/13

Period of this Report: 2/1/14--7/31/14

Report of Receipts and Expenditures

Receipts:

Payments Received to Date: 126,104.00

Total Available for Expenditure: **126,104.00**

	Current Expenditures as of 1/31/14	Cumulative Expenditures	
Salaries and Wages	\$30,945.79	\$50,032.23	
Supplies and Expenses	\$8,932.39	\$24,653.74	
Equipment	\$0.00	\$0.00	
Travel	\$0.00	\$0.00	
Staff Benefits	\$8,863.90	\$13,931.65	
Other - Subcontract			
Subtotal - Direct Costs	\$48,742.08	\$88,617.62	
Indirect Costs - 10%	\$4,874.02	\$8,861.57	
Total Expenditures	\$53,616.10	\$97,479.19	\$97,479.19

Unexpended Balance as of : 7/31/14 _____

\$28,624.81

8/28/14
Date

Valerie Baldwin
Authorized by:
Valerie Baldwin, Manager, ORF