Cystinosis Research Foundation Progress Report

Title: ***Elucidating the role of cystinosin-deficient macrophages in nephropathic cystinosis***

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Progress Report: August 1, 2011 to January 31, 2012

OVERVIEW

The project is well underway and we have started to characterize the macrophage (MΦ) subpopulations within the Ctns-/- kidney. The Ctns null mice are breeding well. We have started our studies on the uninephrectomy Ctns-/- mice to accelerate the rate of fibrosis with some interesting initial findings.

Aim #1: To differentiate the cytokine profile and fibrosis-promoting effects of *Ctns*+/+ and *Ctns*-/- macrophages in mouse models of nephropathic cystinosis and unilateral ureteral obstruction (UUO) induced chronic kidney disease (CKD).

Our overall hypothesis is that *Ctns*-/- MΦ are genetically programmed to execute a more aggressive fibrotic response to renal injury than normal *Ctns*+/+ MΦ that serves an essential role in the pathogenesis of cystinosis-associated CKD. Given that the spontaneous renal phenotype of *Ctns*-/- mice is mild, with fibrosis and impaired glomerular filtration not evident for several months, the proposed studies will be based on two CKD models, comparing outcomes between groups of *Ctns*+/+ and *Ctns*-/- mice on a C57BL/6 background (age and gender matched): (i) Unilateral nephrectomy to accelerate the pace of renal fibrosis; and (ii) UUO-induced CKD.

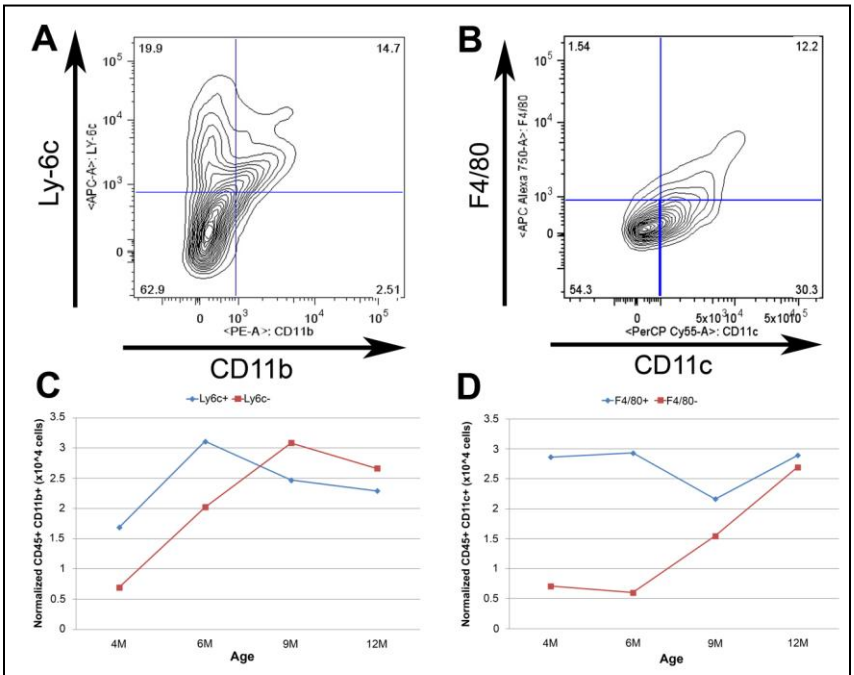

Figure 1 Macrophage subpopulations in nephropathic cystinosis. FACS analysis was performed using the BD LSR II. Kidneys were digested with Liberase into a single cell suspension in FACS buffer and prepared for FACS. After gating for viable, CD45+ cells, analysis for MΦ subpopulations were performed. Representative FACS plot in 12 mos old Ctns-/- mice (A,B); Graphs (C,D) summarize cell populations after normalization. n=1

a) *Accelerating nephropathic cystinosis through uninephrectomy*

Currently, there are no studies characterizing the macrophage subpopulations within the nephropathic cystinotic kidney as disease progresses. Macrophages (MΦ) are a heterogenous population and can

be divided functionally by their phenotype: M1 – classically activated/pro-inflammatory; and M2 – reparative/wound healing, regulatory, and pro-fibrotic. However, distinct M2 markers for these three phenotypes are currently lacking and is an active area of investigation by our lab. We have started with basic macrophage markers: F4/80 – phagocytic MΦ; CD11b – monocytes/ MΦ, neutrophils, and NK cells; CD11c – dendritic/resident tissue MΦ, monocytes, neutrophils, and some B cells; Ly6c – M1 type; CD45 – all myeloid cells.

We have begun to analyze the MΦ subpopulations by FACS analysis (Figure 1). We found that, as expected, the number of MΦ increased with time between 4 to 12 months in Ctns-/- mice, correlating with advancing nephropathic cystinosis. The number of M1-type MΦ ($Ly6c^{med/hi}$) almost doubled between 4 to 6 months and suggests that these cells may initiate a pro-inflammatory response (Fig 1A,C). Interestingly, the level of phagocytic dendritic cells (CD11c+ F4/80+) remained fairly constant during this time period (Fig 1B,D). However, the most dramatic changes appear to be occurring in the $Ly6c^{lo}$ and the CD11c+ F4/80- subpopulations that we speculate may be of the M2 phenotype. We will begin to further characterize these cell populations. These findings will be confirmed in more mice.

Based on studies by Drs. Antignac's and Cherqui's group, Ctns-/- mice begin to develop quantitative signs of nephropathic cystinosis by 9 months. Therefore, we decided to perform a uninephrectomy on 6 month old Ctns-/- mice (n=6/group). Serum was drawn monthly and mice were sacrificed after 3 months (9 months of age). We compared the MΦ subpopulations of 3 month uninephrectomy mice to age-matched controls. We found that there was a greater than 50% increase in the number of M2-type MΦ ($Ly6c^{lo}$) (Figure 2A,B). There was also a more than 50% decrease in phagocytic dendritic cells (F4/80+ CD11c+). We will determine fibrosis severity (total collagen, Picrosirius red staining, myofibroblasts, ECM transcription. We will also check serum creatinine by HPLC and Lipocalin 2 (Ngal). CD11b+ cells have been isolated by AutoMACS from Ctns-/- kidneys and after uninephrectomy.

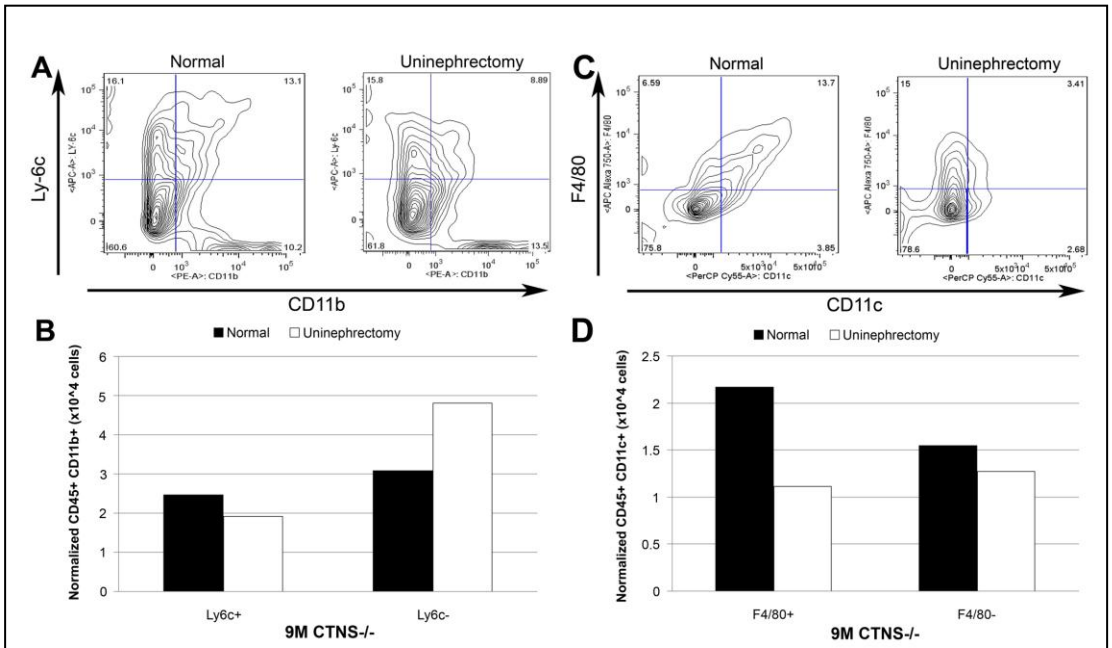

Figure 2 Macrophage subpopulations after uninephrectomy. Uninephrectomy (uniNx) was performed on Ctns-/- mice at 6 months of age and sacrificed after 3 months. UniNx Ctns-/- mice were compared to age matched controls. FACS analysis was performed. Representative FACS plots for 9 month old Ctns-/- mice, (A,C) with graphs below (B,D) that summarize cell populations after normalization. Solid bars – no uniNx; open bars – uniNx; n=1.

b) UUO-induced CKD

Our preliminary data demonstrated that Ctns-/- mice developed more severe fibrosis (19% increase in total collagen) after unilateral ureteral obstruction (UUO) with significantly more F4/80+ MΦ (63% higher). However, qPCR of M1 genes from isolated CD11b+ cells demonstrated a dysregulation in cell signaling (Table 1). We found there was a greater than 15-fold decrease in IL-1β but a 33-fold increase in TNF-α (M1). This could suggest an underlying defect in MΦ signaling/activation (see Aim#2).

	TNF-α	TNF-α R	IL-1β	IL-1β R
Ctns-/- vs. Ctns+/+	34 ± 11*	0.19 ± 0.05*	0.13 ± 0.04*	0.06 ± 0.02*

Table 1 M1 cytokines in CD11b+ macrophages after UUO. *R – receptor; n=7-8; * P<0.01.*

AIM #2. To investigate differences in the cytokine production and fibrogenic responses of *Ctns*+/+ and *Ctns*-/- macrophages in response to apoptotic tubular cells using an *in vitro* model system.

Before continuing to investigate the M1/M2 signaling response to the phagocytosis of apoptotic cells, we will determine whether Ctns-/- MΦ are defective in their response to polarization. Using bone marrow derived MΦ from Ctns-/- and Ctns+/+ mice, we will perform transcriptome analysis using a qPCR array (SABiosciences) after incubation with LPS/IFN-g (M1) and IL-4 (M2). These studies are currently underway.

BUDGET EXPENDITURES

August 1, 2011 - January 31, 2012

Category	Award *	Prior expenditures **	Expenditures this period ***	Anticipated expenditures ****	Total expenditures this period	Total expenditures to date	Remaining balance
Personnel	$ 150,340	$ -	$ 31,955	$ -	$ 31,955	$ 31,955	$ 118,385
Animals /Maintenance	$ 18,270	$ -	$ 1,067	$ -	$ 1,067	$ 1,067	$ 17,203
Supplies	$ 30,450	$ -	$ 9,336	$ -	$ 9,336	$ 9,336	$ 21,114
Travel	$ -	$ -	$ -	$ -	$ -	$ -	$ -
Subtotal	$ 199,060	$ -	$ 42,358	$ -	$ 42,358	$ 42,358	$ 156,702
IDC	$ 19,906	$ -	$ 3,822	$ -	$ 3,822	$ 3,822	$ 16,084
Total	$ 218,966	$ -	$ 46,180	$ -	$ 46,180	$ 46,180	$ 172,786

* Award amount for 08/01/11-7/31/13

** Prior expenditures: Grant began 8/1/11

79% budget remaining

*** Expenditures this period: 08/01/11-01/29/12

*** Anticipated expenditures: 01/30/12-01/31/12