

STRONG CHILDREN'S RESEARCH CENTER

Summer 2015 Research Scholar

Name: Faraz Khan

School: Stony Brook University School of Medicine

Mentor: George J. Schwartz, MD, Jeffrey M. Purkerson, PhD

ABSTRACT

Title: Maturation of innate immune defense mRNA in the collecting duct

Background: It is well known that the distal segment of the nephron is responsible for water regulation and acid-base homeostasis. Intercalated cells (ICs) are a distinct type of epithelial cell in the collecting tubule that mediate acid-base transport and are responsible for acidifying urine. Recent studies suggest ICs are not limited to just acid-base homeostasis, but participate in innate immune defense against urinary tract infection (UTIs). Neonates are particularly susceptible to microbial infections, so understanding the maturation and location of innate immune defense peptides within the collecting duct would facilitate development of improved therapeutic interventions for preventing and treating UTIs.

Objective: To determine the maturation and localization of innate immune defense mRNAs within the collecting duct.

Design/Methods: Collecting ducts were isolated from rabbit kits by DBA-lectin sorting at one and three week time points to determine the maturation of innate immune molecule expression within this nephron segment. We determined relative abundance of innate immune peptide transcripts in the rabbit distal nephron through fold differences in the mRNA expression of neutrophil gelatinase-associated lipocalin (NGAL), Beta Defensin 1 (BD-1), Ribonuclease 7 (RNase 7), and pattern recognition receptor, Toll-Like Receptor 4 (TLR4). Innate immune molecule mRNA abundance was normalized to GAPDH, a housekeeping gene.

Results and Conclusions: When comparing 1 week old kits to a normal adult rabbit, TLR-4, RNase7, NGAL, and BD-1 mRNA abundance was a percentage of normal adult (Table). At day 21, there was an increase in the relative abundance of these peptides. Thus, innate immune defense peptide expression in the collecting duct increases during rabbit maturation.

	TLR-4	RNase7	NGAL	BD-1
Week 1	6%	8%	18%	26%
Week 3	14%	12%	33%	54%

Table: Innate Immune molecule mRNA abundance in CD increases with maturation

A comparison of relative mRNA abundance in DBA-sorted cortex and medulla fragments showed that BD-1 and NGAL expression in medulla was approximately ½ of the level in cortex (BD-1 49%; NGAL 47%); whereas RNase 7 and TLR-4 mRNA were 3- and 11- fold more abundant in medulla, respectively. These results suggest that some innate immune peptides may predominate in the cortex, while others are highly expressed in medulla. A comparison of relative mRNA abundance in PNA-sorted cortex and AE1-sorted medulla showed that BD-1 expression in α -IC enriched cells was roughly 33% of the level expressed in β -IC enriched cells; whereas NGAL, TLR-4, and RNase 7 were 1-, 1.6-, and 3.3- fold more abundant in α -IC enriched cell population, respectively. These data indicate that although both IC subtypes express these mRNAs, α -IC in the medulla may be more effective in innate immune defense during UTIs. Lastly, NGAL and BD-1 mRNA abundance in a PNA lectin-sorted cells isolated from kidney cortex was 3.4- and 5.9-fold higher, respectively in acidotic rabbits compared with normal, suggesting acidosis enhances innate immune defense in the collecting duct. This novel finding will require further investigation at the protein and immune defense level.