STRONG CHILDREN'S RESEARCH CENTER

Summer Research Scholar

Name: Coralee Everett

School: University of Rochester

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

ABSTRACT

Title: Dietary Ammonium Supplementation Compromises NOS-Dependent Innate Immune Defense

Background: Acute pyelonephritis is a common kidney bacterial infection in children. Uropathogenic E. coli (UPEC) causes >70% of these urinary tract infections. Metabolic acidosis is often associated with severe pyelonephritis. Ammonium chloride (NH_4Cl) or HCl supplementation induce metabolic acidosis; however, only NH_4Cl increases UPEC burden and exacerbates pyelonephritis.[1] Dietary ammonium is excreted as urea nitrogen via urea cycle in which Arginases convert L-Arginine to urea and ornithine, the latter being a precursor to polyamine synthesis. Polyamines have been reported to mediate resistance to nitrosative stress[2] and increase UPEC fitness.[3]

Objective: To determine whether dietary ammonium impairs iNOS/NOS2-dependent innate immune defense by limiting L-arginine substrate, thereby increasing polyamine/NO ratio.

Methods: Female C3H-HeN (Tlr4-sufficient) and C3H-HeJ (Tlr4-deficient) mice 5-7 weeks of age were fed normal rodent chow (NC), 2% ammonium chloride supplement (MA), or administered 2.3mg/kg/day BEC, 25mg/kg/day DFMO, and 15mg/kg/day MDL-72,527 via ALZET® pump. UPEC (CFT0073) was injected transurethrally. UPEC-UTI burden (cfu/g) was determined in bladder and kidney tissue homogenates. Urine NO and polyamine metabolites (uM) were measured using colorimetric and fluorimetric assays, respectively, and urines were normalized for osmolality. Bladder and kidney NOS2 mRNA abundance were quantitated by qRT-PCR. Statistics: Two-tailed T-test or Mann-Whitney U-Test, $p<0.05$.

Results: NH_4Cl supplementation in HeN mice exacerbated UPEC-UTI (Median: NC=251; MA=229730; $p<0.001$) and led to 70% decrease in urine NO metabolites (Mean±SE: NC/NCavg=1.0±0.2; MA/NCavg=0.3±0.1; N=6; $p<0.05$) despite elevated NOS2 mRNA expression (NOS2 fold±SE: MA-HeN=8.7±1.8, N=10, $p<0.001$). NC-HeN mice urine NO metabolites increased 20% post-infection consistent with NOS2 induction, whereas NC-HeJ mice NO metabolites were unchanged (Mean±SE: HeN-Uninf=3431±86; HeN-Inf=4152±63; HeJ-Uninf=1228±251; HeJ-Inf=1154±279; 5 mice/group), demonstrating Tlr4 dependence. Polyamine synthesis inhibition increased NO metabolites 40% (Mean±SE: 4946±94; 5 mice/group) whereas NH_4Cl supplementation increased polyamine/NO ratio 6.5 fold (Ratio: NC-HeN=1.4, MA-HeN=9.1), indicating competition between NOS and Arginase for L-arginine substrate.

Conclusions: Induction of NOS2 expression and NO synthesis are components of the Tlr4-dependent innate immune response. NH_4Cl supplementation in Tlr4-sufficient mice exacerbates UPEC-UTI burden and shunts arginine metabolism down the Arginase pathway, thereby increasing the polyamine/NO ratio.

References:

1. Purkerson JM, Corley JL, Schwartz GJ. Metabolic acidosis exacerbates pyelonephritis in mice prone to vesicoureteral reflux. Physiol Rep. 2020 Oct;8(19):e14525. doi: 10.14814/phy2.14525. PMID: 33030238; PMCID: PMC7543054.
2. Bower, J. M., & Mulvey, M. A. (2006). Polyamine-Mediated Resistance of Uropathogenic Escherichia coli to Nitrosative Stress. *Journal of Bacteriology*, *188*(3), 928–933. https://doi.org/10.1128/jb.188.3.928-933.2006
3. Bower, J. M., Gordon-Raagas, H. B., & Mulvey, M. A. (2009). Conditioning of Uropathogenic Escherichia coli for Enhanced Colonization of Host. *Infection and Immunity*, *77*(5), 2104–2112. https://doi.org/10.1128/iai.01200-08