Cytotoxic Necrotizing Factor-1 Toxin in Uropathogenic E. coli

Is There a Role for Virulence Factors in Urinary Tract Infection?

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Acknowledgements

• This work was supported in part by NIH grants, NS26310 and AI84984

• The authors declare no conflict of interest
Background

• UTI is common in children, accounting for 1 million visits to the pediatrician yearly (NIH)
• Incidence of UTI in children <6 years old:
  – 3-7% in girls
  – 1-2% in boys
• Risk of recurrence is 40-60%
• Studies on pediatric UTIs are important because of risk of hypertension, chronic kidney disease
Prevalence of UTI in the Pediatric Population

Etiology of Neonates Admitted for Serious Bacterial Infection

- UTI
- Bacteremia and UTI
- Bacteremia
- Bacteremia and meningitis
- Meningitis
- Abscess
- Other

Byington C, Rittichier K, et. al. Pediatrics 2003;111;964
Age Distribution and Renal Scarring in Patients with Pyelonephritis

Renal Consequences of Pyelonephritis

No. of Patients

Scar
No Scar

No. of Patients

Age at Diagnosis of Pyelonephritis

0 2 4 6 8 10 12 14 16
Challenges

- Rising incidence of antibiotic-resistant bacteria
  - Widespread use of antibiotics
  - Pathogenesis of UTI is multifactorial
  - Host and bacterial phenotypes play critical roles

- Therefore, it is important to be able to examine bacterial pathogenicity in a variety of ways, including in vivo models
E. coli

- Most common pathogen in UTI
  - 50-80% of all culture-proven UTIs
- UTI-associated pathogenic E. coli (UPEC) isolates express cytotoxic necrotizing factor 1 (CNF-1)
  - CNF-1 expressing E. coli have been shown in vitro to release proinflammatory cytokines and to exhibit resistance to host neutrophil and monocyte phagocytosis
cnf-1 and hly are inherited as a pathogenicity island and are part of the prs operon
CNF-1

- Found in strains of *E. coli* implicated in UTI and neonatal meningitis
- CNF-1 belongs to a group of cytotoxins that cause activation of Rho guanosine triphosphatases (GTPases)
  - Alters host cell actin cytoskeleton
  - Promotes bacterial invasion of endothelium that comprises blood-brain barrier

• However, there have been conflicting reports on the exact role of the *E. coli* cytotoxic necrotizing factor-1 (CNF-1) in the pathogenesis of UTI
Goals

- Establish a murine model for examination of uropathogenic *E. coli* in the urinary tract
- Elucidate the role of CNF-1 in UTIs
Materials/Methods

• Targeted deletion of cnf-1 in the isolate of UPEC (cnf-1-null strain)

• Comparative studies in several mouse strains, with transurethral inoculations of wild-type or cnf-1-null strain UPEC
  – 1-2 × 10^7 CFU/ mouse in 50 µl of PBS
  – Urine samples were collected and plated to verify pre-inoculation sterility

• At various time points, kidney and bladder tissues were harvested and analyzed for inflammation and bacterial burden
Murine Model of Cystitis and Pyelonephritis

Transurethral *E. coli* Inoculation

Outcomes

Bacterial Burden

Inflammatory Response

Kidney and bladder bacterial cultures

Pathology and Flow cytometry
Results

• Bacterial counts were consistently present in infected mice, and absent from control mice (PBS)

• Both the wild-type UPEC strain and the cnf-1 null strain caused robust and reproducible bladder and kidney infections
Bacterial burdens of mice infected with wild-type or \textit{cnf-1}-null \textit{E. coli} were not significantly different.
Examination of Inflammation in Murine UTI

Preliminary data show no alteration in inflammation with cnf-1-null bacteria
Conclusions

• A role for CNF-1 in the pathogenesis of UTI is often inferred from the prevalence of CNF-1 expression in clinical isolates of UPEC
• Our preliminary data casts doubt on the clinical virulence of CNF-1
• Additional studies are in place to study potential genetic linkage of cnf-1 with other factors that might increase UPEC clinical virulence
Acknowledgments

Department of Urology
Jason E. Michaud, MD, PhD
William Harty, BS

Pediatric Infectious Disease
Kwang Sik Kim, MD
Donna Pierce

Thank You!
References


