Washington University

Theme: Molecular and epidemiologic basis of acute and recurrent urinary tract infections (UTI's) in women

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Abstract

This interdisciplinary SCOR program seeks to understand the epidemiology, pathogenic strategies, and resultant host responses of urinary tract infections (DTI) caused by uropathogenic E. coli (UPEC), one of the most common diseases affecting women. This knowledge will be applied to critically evaluate all aspects of clinical UTI management, including diagnosis, treatment, and prevention. We have proposed an integrative and translational set of experiments that capitalizes on the complementary expertise found in each of the three projects. Basic scientists in Projects 1 and 3 have access to uropathogenic strains collected from women in Project 2 at different clinical stages of UTI. Working together, Projects 1, 2, and 3 will identify genetic and molecular markers and correlates of the different clinical UTI syndromes associated with UPEC infection. These will be pursued both in humans (Project 2) and mice (Project 1) for prognostic indicators of disease outcome: bacterial clearance, asymptomatic infection, chronic colonization, or recurrence. Genotypic and phenotypic profiles of UPEC strains from well-characterized UTI cases will be generated in Project 1 and 3 by blending a powerful genetic system with functional and comparative genomics, defined in vitro and murine models, comparative immunoproteomics, biochemistry, cell biology, laser capture microdissection, antigen discovery techniques, and high resolution electron microscopy. The host response to intracellular bacterial communities (IBCs) and quiescent intracellular reservoirs (QIRs) formed by different UPEC isolates will be examined in detail both in a mouse model (Project 1), using gene and cytokine expression profiling, and in humans (Project 2), by monitoring the adaptive immune response and metabolite profiles in human urine. In addition, exfoliated bladder epithelial cells in mouse and human urine will be screened for evidence of IBC formation, allowing parallel correlation of microscopic assays with clinical outcome in both mice and

humans. These efforts promise to connect specific measurements made at the bench to clinical outcomes observed at the bedside. Project 3 will also address primary prevention of UTI by using comparative pan-genomics to study the mechanism by which UPEC emerge from the distal gastrointestinal tract and traverse the perineum to the urethra to cause infection. Completion of these interwoven projects promises to address questions in the clinical management of this ubiquitous disease.

Project 1: Host-Pathogen Interaction in Acute and Chronic Urinary Tract Infections
Type: Basic

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Abstract

Urinary tract infections (UTI) caused by uropathogenic Escherichia coli (UPEC) result in 8 million outpatient visits (mostly by women) and over $1 billion in health care costs annually in the U.S. Recurrent UTI (rUTI) is a significant problem: >25% of women who suffer from an acute UTI experience a rUTI within 6 months. To address this problem, Project 2 is enrolling a cohort of women presenting with acute cystitis but no recent history of UTI. Bacterial isolates from the urine of these patients will be characterized in our mouse model in Project 1. We have detailed a pathogenic cycle within mice in which UPEC invade superficial umbrella cells that line the bladder and rapidly replicate to form a densely packed, biofilm-like intracellular bacterial community (IBC). Bacteria within an IBC are protected from both host innate immune defenses and many antibiotics, thus allowing one bacterium to clonally replicate to 10,000 or more bacteria within hours after infection. IBCs eventually disperse, with bacteria fluxing out of the cells and going on to repeat the IBC cycle within another epithelial cell or going on to form a quiescent intracellular reservoir (QIR). QIRs can persist for months and then later seed a rUTI. We will determine whether IBC and QIR formation is a general property of UPEC and whether prominent features of these cycles differ between bacteria causing different clinical UTI syndromes (asymptomatic bacteriuria, acute infection, or recurrent infection) and between different host genetic backgrounds. We have completed the genome sequence of cystitis strain UTI89 in Project 3 and will use this with additional sequence information to computationally search for candidate genetic determinants of pathogenesis and recurrence. These candidate genes will be analyzed in our murine model, in part by investigating the effects of mutations using advanced imaging methods, laser capture microdissection, functional genomics, and gfp fusions. Host responses will be characterized by immunohistology, flow cytometry, and cytology. Mass spectrometry will be used to profile qualitative and quantitative changes in both host and pathogen factors to identify those with pathophysiologic and prognostic significance. The combined expertise of the three projects in this SCOR will allow a coordinated effort to

understand the epidemiology and basic pathogenic mechanisms of UTIs in women, allowing us to begin addressing clinical questions in the management of UTI.

Project 2: Host-Response to Recurrent Urinary Tract Infections in Women
Type: Clinical

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Acute uncomplicated urinary tract infections (DTI) occur in an estimated 7-11 million women each year at a cumulative cost estimated to exceed $1 billion annually. Approximately 20-30% of women suffer from frequent recurrent infections. UTI in young women result in substantial morbidity, time lost from work, and medical costs. An improved understanding of the pathogenic mechanisms underlying UTI could result in new diagnostics and novel approaches to prevention and treatment resulting in tailored therapies, decreased antimicrobial use, and decreased morbidity. The goal of this project is to prospectively follow a well defined group of patients from acute to recurrent UTI and obtain clinical samples to better understand UTI pathogenesis. The clinical samples will be analyzed throughout this and the accompanying 2 projects to understand the ecological niches in which uropathogens reside and persist, the host responses to the primary acute infection and persistence, and uropathogen virulence repertoires that dictate the interplay between pathogen and host resulting in different clinical syndromes such as acute cystitis and asymptomatic bacteruria. In this project, we will prospectively follow a large cohort of women from single isolated acute to recurrent UTI to determine 1) the innate immune response to infection in patients who have a single isolated UTI vs. those with recurrent infection, differentiating same strain and different strain recurrence and correlating elements of the innate response with urovirulence characteristics of the infecting strain, 2) the adaptive response to infection of patients who have a single acute event vs. those with recurrent infection and 3) the association of intracellular bacterial communities (IBC) and IBC derived factors with recurrent UTI. Through translation from clinical to basic molecular studies, this project will identify unique host responses to same strain and different strain recurrent UTI. Uropathogenic strains isolated from well-characterized episodes of UTI and asymptomatic bacteriuria will be used in Project 1 for comparative genomics to identify and test the virulence repertoire necessary for different subtypes of UTI. The molecular pathogenesis of representative strains and the contribution of specific virulence factors to disease will also be examined in Project 1. Uncultured genitourinary tract samples will be evaluated for the evolution of the microbial ecology from acute to recurrent UTI in Project 3. Together these studies will bring together key

elements of UTI epidemiology and molecular pathogenesis to develop optimal management and preventative strategies.

Proejct 3: Pan-genome of E. coli in Bladder and Gut of Women with Recurrent Urinary Tract Infection
Type: Basic

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Abstract

The unexpectedly high diversity and rate of evolution of UPEC are contributing to the difficulty in understanding molecular mechanisms underlying recurrent UTI. Advances in sequencing, algorithm development, and computing power provide an opportunity to elucidate the genetic and molecular basis for recurrent UTI using comparative genomics. The work proposed in Project 3 for the next funding period is designed to capitalize on the complementary expertise and biospecimen resources available in this SCCOR program. Gut and urine-associated E. coli strains recovered from three patients with recurrent UTI, enrolled in the clinical trial described in Project 2, will be selected and sequenced using the highly parallel 454 pyrosequencer. The sequence data will be used to examine E. coli evolution within a given individual, in two host habitats, over the course of three separate episodes of infection, and between different individuals. Follow-up studies will involve (i) quantitation of gene prevalence in isolates recovered from the two ecosystems (using new comparative pan-genomic methods and PCR-based sequence surveys), (ii) resequencing of genes statistically enriched in urine isolates to determine, based on maximum likelihood and parsimony algorithms, whether they are under positive selection in UPEC strains; and (iii) performing functional annotations. Genes that are (i) more prevalent in urine isolates than in fecal isolates within individual patients; (ii) more prevalent in UPEC strains than non-UPEC strains in a broad panel of clinical isolates; (iii) under positive selection in UPEC strains; and (iv) have functional annotations suggestive of a role in pathogenesis will be tested in a mouse model of UTI in collaboration with Project 1.

CORES

Administrative Core

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Abstract

The SCOR Program Director, Dr. Scott J. Hultgren, Ph.D., will supervise the Administrative Core at Washington University. He will be responsible for the overall administration of the SCOR, including scientific direction, fiscal policy, personnel actions, interactions with consultants, collaborators and subcontractors, seeking the advice of outside experts, organization of regularly scheduled research meetings and interactions with the granting agency.

Coordination of Scientific effort: While each of the project P.l.s will have responsibility for the scientific direction and budget oversight of their respective projects, scientific progress and budgetary considerations will be discussed by each project P.I. with the Director, Dr. Hultgren. To facilitate this, Dr. Hultgren will meet with or contact each of the other project P.l.'s at least monthly. To further facilitate the basic science research involved members of the Hultgren and Gordon laboratories will meet twice a month to discuss the research. To facilitate the coordination of projects Dr. Hultgren will meet at least twice each calendar year with Dr. Walter Stamm (once at Washington University in St. Louis and once at the University of Washington in Seattle). As needed other members of each of the three teams of researchers will meet as a group to discuss results. In addition, we forsee a need for much more extensive data management to make this program fully accessible to program members and the general research community so we anticipate seeking additional funding for broader data management resources. The guiding principles behind design of such a system will be: (i) protection against data loss; (ii) maximization of data value; and (iii) active control of data privacy and security, particularly in research involving human subjects Each of these principles has two aspects. Data loss includes both physical loss (hardware failure resulting in loss of data files) as well as functional data loss (data that is unable to be accessed and used because it is in a nonstandard format, is in a noncentral location, or is not properly characterized with experimental metadata). Data value is measured both in terms of present and immediate value (answering the question for which the experiment was done) and in terms of future potential value (for instance, proactive organization and archival storage of microarray data increases each array's value in the future for potential meta-analyses). Control of data privacy and security refers generally to data separation. Development and publicly accessible networks and computers must be separated and isolated to protection against unauthorized access and modification. Data itself must also be categorized and isolated so that privacy concerns are appropriately addressed without causing extension of access prohibition. In other words, the restrictions should be appropriate, neither too liberal nor too draconian, allowing maximal data sharing with maximal privacy and security protection. Clinical

databases at the University of Washington include both clinical and epidemiological data from study patients and microbial data currently maintained in bacterial strain repositories. Extensive data of both types from the currently funded period and from anticipated future projects have been accumulated and need to be consolidated and managed in a single database management system.