URINARY TRACT INFECTION: HOW IT HAPPENS?

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ABSTRACT

Urinary tract infections (UTIs), including cystitis and pyelonephritis, affect a large proportion of the world population and account for substantial morbidity and medical costs. Classification of the UTIs is based on the anatomical level of infection, the grade of severity of infection, the underlying risk factors, and the microbiological findings. Uropathogenic *Escherichia coli* is the causative agent in 70-95% of community-acquired uroinfections and about 50% of all cases of nosocomial uroinfections. Virulence factors associated with uropathogenic strains of *E. coli* contain toxins such as haemolysin and cytotoxic necrotising factor, capsules, lipopolysaccharide, the siderophore aerobactin, and adhesive organelles. The ability to attach to urothelial cells is the most important determinant of pathogenicity. An adherence is followed by inflammation involving the urothelial cells' cytokine response. Whereas interleukin (IL)-6 can cause the fever and systemic response of the UTIs, IL-8 can function as a neutrophil chemoattractant. Cytokines released by T cells and monocytes modify initiative urothelial cells' cytokine response to bacteria. Nevertheless, antibiotic treatments can effectively sterilise the urine, but bacteria can survive and persist in the bladder tissue, serving as a reservoir for the recurrent UTIs. The severity of UTI reflects the quality and magnitude of the host response. While strong local and systemic innate immune activation occurs in patients with acute pyelonephritis, the response to asymptomatic bacteriuria is low. It should be reasonable to 'individualise' diagnosis and therapy by interconnecting information on uropathogenic bacterial virulence and the host response.

Keywords: Urinary tract infection, *Escherichia coli*, virulence, interleukin.

INTRODUCTION

Urinary tract infections (UTIs) are assumed to be the most common bacterial infections accounted in the United States for approximately 7 million office visits, 1 million emergency department visits, and >100,000 hospital admissions annually, most often for acute pyelonephritis. A diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings a diagnosis is based on dysuria, frequency, urgency, and suprapubic pain. These lower urinary tract symptoms are classically present in cystitis, whereas pyelonephritis is associated with fever, chills, and flank pain. Women are more likely to experience UTI than men. Almost half of all women will experience one UTI episode during their lifetime. Specific subpopulations at increased risk of UTI include pregnant women, infants, the elderly, and patients who have diabetes, underlying urological abnormality, urinary catheter, spinal cord injury, multiple sclerosis, and immunodeficiency syndrome. Catheter-associated UTI is the most common nosocomial infection, accounting for >1 million patients in hospitals and nursing homes. The risk of UTI increases with duration of catheterisation. In nonobstructed and non-pregnant women, acute uncomplicated UTI seems to be a benign disease with no long-term sequelae. Nevertheless, UTI increases the risk of premature delivery and foetal mortality among pregnant women as well as pyelonephritis, impaired renal function, and end-stage renal disease among paediatric patients. The estimated annual cost of community-acquired UTI is significant, costing approximately $1.6 billion in the United States.[1]

ASCENDING ROUTE OF INFECTION

UTIs represent the inflammatory response of the urothelial cells to bacteria mostly entering from the bowel reservoir via an ascending route through the urethra into the bladder. This route is further enhanced by significant soilage of the perineum with faeces, utilisation of catheters, or spermicidal agents.[2,3] Indwelling catheters with open-drainage systems result in bacteriuria in almost 100% of cases within 3-4 days. The more compromised natural defence mechanisms with, for example, urinary obstruction or catheterisation, the fewer the virulence requirements of any bacterial strain to induce UTI. Although cystitis is restricted to the bladder, approximately 50% of UTIs can extend into the upper urinary tract. The most episodes of pyelonephritis are caused by retrograde ascent of bacteria from the bladder through the ureter to the renal pelvis and parenchyma. Although reflux of urine is probably not required for ascending infections, oedema associated with cystitis may cause sufficient changes in the ureterovesical junction to permit reflux. Once introduced into the ureter the bacteria may ascend to the kidney, and this ascension would be considerably facilitated by any process that interferes with the regular ureteral peristaltic functioning. Gram-negative bacteria and their endotoxins, as well as pregnancy and ureteral obstruction, have a significant antiperistaltic effect. Bacteria that reach the renal pelvis can enter the renal parenchyma by means of the collecting ducts at the papillary tips and then ascend upward within the collecting tubules. This process is accelerated by elevated intrapelvic pressure from ureteral obstruction or vesicoureteral reflux as well as in the presence of intrarenal reflux.

The vast majority of UTIs are caused by bacteria usually originating from the bowel flora. *Escherichia coli* is the most common uropathogen, responsible for over 80% of community-acquired and approximately 50% of hospital-acquired UTIs. Other Gram-negative Enterobacteriaceae such as *Klebsiella* and *Proteus* as well as Gram-positive *Enterococcus faecalis* and *Staphylococcus saprophyticus* are responsible for some of the other community-acquired UTIs. Nosocomial infections are caused by *E. coli*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Serratia*, *Pseudomonas aeruginosa*, *E. faecalis*, and *Staphylococcus epidermidis*. Uropathogens such as *S. epidermidis* and *Candida albicans* originate from the flora of the vagina or perineal skin.[4,5]

Bacterial Adherence

Uropathogenic E. coli (UPEC) can infect the urinary tract not by chance but rather by the expression of virulence factors that enable them to adhere to and colonise the perineum and urethra and migrate to the urinary tract, where they establish an inflammatory response in the urothelial cells (Table 1).[6] Genomic analysis of a UPEC strain revealed the presence of genes for putative chaperone/usher systems such as autotransporter proteins that may function as motility mediators, adhesins, invasins, proteases, and serum resistance factors.[7] Autotransporter serine protease Sat may cause cytoplasmic vacuolation and severe histologic damage.[8] Alpha-haemolysin forms pores in the urothelial cell membranes.[9] UPEC produces several iron acquisition systems and iron system. Most UPEC strains generate an acid polysaccharide capsule that protects the bacteria from phagocytosis by human polymorphonuclear leukocytes and inhibits activation of complement.[10] UPEC expresses a number of adhesins that allow attachment to the urothelial cells.[11] Bacteria assemble adhesins on their surface as monomers, oligomers, or supramolecular fibres called fimbriae or pili. The adhesive organelles that are associated with UPEC strains include S pili, Dr family adhesins, P pili, and Type 1 pili.[12] S pili recognise sialyloligosaccharide residues on urethelial cells and can help colonisation of the upper urinary tract. Dr family adhesins bind the Dr^a blood group antigen present on decay accelerating factor. P pili bind the α-D-glucopyranosyl-(1-4)-β-D-galactopyranoside moiety present in the globoseries of glycolipids that are expressed by erythrocytes and kidney endothelial cells. Therefore, P pili act as a major virulence factor of pyelonephritis. Type 1 pili are the most widely distributed among UPEC strains.[13] Type 1 pili consist of a 7 nm thick helical rod made up of repeating FimA subunits connected to a 3 nm wide distal tip fibrillar structure containing two adapter proteins FimF and FimG joined to the adhesin FimH.[14] FimH binds mannose containing glycoprotein receptors and may mediate UPEC attachment.[15] The FimH adhesin constitute a COOH terminal pili domain involved in the incorporation of FimH into Type 1 pili and an NH_3 terminal adhesin domain consisting carbohydrate binding pocket capable of receiving a D-mannose.[16] Interaction of the FimH with glycoprotein receptor on the bladder uroepithelial cell is a crucial step of the UPEC colonisation and the onset of the cystitis.[13,16,17]

Table 1: *Escherichia coli* virulence factors.

Fimbriae/adhesins
- Type 1, Type 3, P-fimbriae, S-adhesin family, Afa/Dr adhesin family, Curli
Toxins
- α-haemolysin, cytotoxic necrotising factor 1
Autotransporter adhesins
- Antigen 43, UpaG
Autotransporter serine proteases
- Sat, Vat
Iron acquisition systems
- Enterobactin, Aerobactin, Yersiniabactin, Salmochelin, Iha, Haem receptors Hma, ChuA
Extracellular polysaccharides
- cellulose, poly-β-1,6-N-acetyl-D-glucosamine
Flagella
Capsule
O antigen

The luminal surface of the inner bladder wall is covered by 3-4 layers of a stratified urothelium. A thin basement membrane and lamina propria divide the urothelium from the smooth muscular and serous layers of the outer bladder wall. The urothelium contains small-size undifferentiated basal and intermediate epithelial cells underlying a single layer of large-size highly differentiated multinucleate luminal facet cells known as umbrella cells. These umbrella cells deposit on their luminal surfaces uroplakins as a quasi-crystalline array of hexagonal complexes holding four integral membrane proteins.[18] Uroplakins UPIa, UPIb, UPII, and UPIII cover almost entirely the luminal surface of a bladder. An asymmetric unit membrane (AUM) as thick luminal uroplakin-embedded membrane is a permeable barrier that strengthens and stabilises facet cells, preventing a bladder wall from rupturing after accumulating a considerable volume of the urine. Type 1 pili of the UPEC can specifically bind to uroplakins UPIa and UPIb as a first step of bacterial invasion.[19] This binding can be inhibited by enzymatic deglycosylation of UPIa nad UPIb or by D-mannose as the soluble FimH receptor analogue.[20,21] Therefore, the FimH-containing tips of Type 1 pili mediate bacterial attachment to the uroplakin-embedded AUM of the facet cells lining the bladder lumen. Bacteria attach to the grooves and niches formed by the AUM of facet cells singly and in large biofilm-like colonies.[15]

Bacterial Invasion

The facet cells lining the luminal surface of the bladder internalise bacteria that are observed free within the cytoplasm and within membrane-bound vacuoles.[22] The AUM of the luminal facet cells may zipper around and envelop adhered bacteria via interactions with Type 1 pili.[15] FimH can function as an invasin and internalin A in such a manner that FimH mediated bacterial invasion of bladder urothelial cells requires the activation of signal transduction cascades including protein tyrosine kinases, phosphoinositide-3 kinase, and actin cytoskeletal rearrangements.[23] Moreover, FimH mediated invasion correlates with the formation of complexes between adhesin kinase and phosphoinositide-3 kinase and between the cytoskeletal components α-actinin and vinculin. These occurrences contribute to the modulation and stabilisation of actin cytoskeletal alterations that can lead to envelopment and internalisation of UPEC subsequent to FimH mediated bacterial adherence.

A micturition can work to wash out nonattached or weakly attached bacteria from the bladder urothelium.[12] The low pH and osmolarity of the urine can be inhibitory factors to bacterial growth. The salts, organic acids, and urea from the urine can reduce bacterial survival in the bladder. A lactoferrin present in urine can scavenge essential iron away from incoming bacteria. Additionally, Tamm-Horsfall protein, secretory immunoglobulin A, uromucoid, and low molecular weight sugars can act as anti-attachment factors competitively inhibiting bacterial adherence to the bladder urothelium. A continued presence of bacteria within the bladder can trigger the activation of additional local defence mechanisms.

Neutrophil Recruitment and Cytokines

Exfoliated bladder cells associated with bacteria are often found in the urine of patients having UTIs.[22] Such clearance of infected and damaged bladder cells can function as a defence mechanism. The FimH mediated bacterial adhesion and afterwards invasion is crucial in the induction of bladder urothelial exfoliation during an UTI. Bladder cell exfoliation as a response to UTI with Type 1-piliated *E. coli* occurs via an apoptosis-like mechanism including DNA fragmentation and an activation of proteolytic enzymes known as caspases.[15] These cysteine proteases are critical components in the initiation and execution of apoptotic pathways.[24] Rather than directly triggering the exfoliative process, FimH mediated bacterial adhesion and invasion can serve to deliver other bacterial virulence factors such as lipopolysaccharide (LPS). Within 6 hours of Type 1-piliated UPEC attachment and invasion with obvious bladder cell exfoliation, neutrophils may be seen entering the bladder urothelium and lumen. Neutrophils and macrophages provide the first line of defence of the innate immune system by phagocytosing, killing, and digesting bacteria. Killing is accomplished by digestive enzymes and by oxygen free radicals and other reactive oxygen species, generated by the NADPH oxidase, and oxidised halides produced by myeloperoxidase. The oxidase pumps electrons into the phagocytic vacuole, which produces conditions conductive to microbial killing and digestion by enzymes released into the vacuole from the cytoplasmic granules. This process is greatly enhanced by the complement proteins that not only are cytotoxic but are also attractants for phagocytic cells and facilitate inflammation and cell adhesion. The combination of innate and adaptive immunity is required for complete eradication of the offending organisms.

Neutrophils or polymorphonucleocytes (PMNs) are phagocytic inflammatory cells that may mediate bacterial killing through the creation of reactive oxygen intermediates and release of preformed antibacterial peptides. Neutrophil recruitment is critical for bacterial clearance from the urinary tract, therefore the pyuria (presence of neutrophils in the urine) is a hallmark of UTI.[25] Interactions between the neutrophil receptor CD11b/CD18 (Mac-1) and the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) on the bladder urothelium have been shown to be crucial for neutrophil influx into the urothelium. Thus, a bacterial infection induces the expression of ICAM-1 by the bladder urothelium.[26] Elucidation of the molecular mechanisms included in neutrophil recruitment into the urinary tract has highlighted the importance of cytokines and chemokines. These soluble molecules are produced in response to a variety of different agents (such as LPS) and may regulate the inflammatory process. In patients with UTIs the cytokine interleukin (IL)-6 and chemokine IL-8 are present in the urine, thereby suggesting that urothelial cells seem to be a major source of IL-6 and IL-8 following UPEC infection.[27,28] IL-6 is a pleiotropic cytokine with different immunoregulatory functions such as amplification of signals included in a neutrophil recruitment.[29,30] Urine IL-6 values from patients having UTIs correlate with disease severity.[31] IL-8 is a member of the CXC chemokine family and a potent neutrophil chemotactic molecule, therefore the induction of IL-8 following UPEC infection correlates with the presence of neutrophils in the urine.[32] Type 1 and P-piliated UPEC strains induce considerably more cytokines than their nonpiliated isogenic counterparts.[33] P pili may activate urothelial cytokine production via a ceramide and serine/threonine kinase-dependent signalling pathway.[34] Type 1 pili indirectly activate urothelial IL-6 production by mediating bacterial internalisation.

BACTERIAL VIRULENCE VERSUS HOST SUSCEPTIBILITY

Bacterial virulence factors influence the site and severity of UTIs. A general background of UTI pathogenesis is based on the mechanisms of UTI susceptibility, with a particular focus on genetic variation affecting innate immunity. The innate immune response (IIR) of the host is critically important in the antibacterial defence mechanisms of the urinary tract and bacterial clearance normally proceeds without sequelae. The symptoms of acute pyelonephritis are caused by the IIR, therefore an inflammation in the urinary tract decreases renal tubular function and may lead to renal scarring, particularly in childhood. On the other hand, in children with asymptomatic bacteriuria (ABU) uropathogenic bacteria persist without causing symptoms or any pathology. ABU strains are phylogenetically related to strains that cause symptomatic UTI. Most ABU strains adhere poorly to epithelial cells, but identified is a subgroup of strongly adherent strains that are unable to stimulate an epithelial cell IL-6 cytokine response.[35] Invading bacteria trigger a response determined by their virulence factors, mediating

adherence to the urothelial cells by means of signalling through Toll-like receptors (TLRs) and activating the defence mechanisms. In ABU strains such virulence factors are in the majority of cases not expressed. Thereby genetic alterations that reduce TLR4 function are associated with ABU, while polymorphisms reducing interferon regulatory factor 3 (IRF3) or CXC chemokine receptor 1 (CXCR1) expression are associated with acute pyelonephritis and consecutive renal scarring. The IRF3-dependent signalling pathway is critical for distinguishing uropathogens from normal flora at the urothelial barrier. TLR4 signalling was initiated after ceramide release from glycosphingolipid receptors through TRAM, CREB, Fos, and Jun phosphorylation and p38 mitogen-activated protein kinase-dependent mechanisms following with nuclear translocation of IRF3 and activation of IRF3/interferon beta-dependent antibacterial effector mechanisms. This TLR4/IRF3 pathway of uropathogen discrimination is activated by ceramide and by P-fimbriated UPEC that use ceramide-anchored glycosphingolipid receptors. The relevance of this pathway was supported by polymorphic IRF3 promoter sequences differing between children with severe acute pyelonephritis and children who are asymptomatic bacterial carriers.[36-38]

RECURRENT UTIs

The UPEC survival in the urinary tract depends on the infected bladder epithelial cells' exfoliation and the neutrophils' influx. The urothelial cells production of antibacterial factors such as nitric oxide and defensins, together with activation of immune cells like mast cells and macrophages, may help to control UTIs. In addition to both innate and adaptive defence mechanisms is the possible dissemination of UPEC within the urinary tract and persistance within bladder urithelial cells for days or even weeks. Antibiotic treatment can effectively reduce an amount of bacteria in the urine, but still UPEC strains can persist within bladder tissue.[39] UPEC is capable of fluxing into and out of the bladder urothelial cells. It means that invading uropathogens may multiply and afterwards escape out of the urothelium before the exfoliation process is completed. UPEC can persist for a long period of time in a quiescent state, serving as a source for recurrent UTIs, and can still be undetectable in the urine.

ANTIBIOTIC RESISTANCE

UTIs are responsible for a considerable portion of antibiotic use, thus representing a significant social and economic burden. Widespread use and extensive misuse of antibiotics directly correlates with the emergence of antibiotic resistance. The World Health Organization has recently issued a factsheet emphasising the worldwide dramatic increase in antibiotic resistance. In light of a very serious public health problem, the World Alliance against Antibiotic Resistance has proposed a 10-point action plan to combat the rapid rise of worldwide antibiotic resistance. UTI may present clinically as benign, uncomplicated cystitis or severe, life-threatening urosepsis as well as urethritis or multidrug-resistant tuberculosis. Due to the heterogeneity of UTIs, the European Association of Urology/European Section of Infections in Urology introduced the ORENUC classification system based upon the clinical presentation, categorisation of risk factors, and the antibiotic susceptibility of the causative uropathogens. In the scenario of urosepsis, early diagnosis and a vigorous therapy are mandatory. Where appropriate, a successful decompression of the obstructed urinary tract is crucial for survival.[40,41]

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