



Molecular Defense Mechanisms during Urinary Tract Infection

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The urinary tract is a common site of infection. The complete mechanisms of urinary tract infection (UTI) are still unknown. In general, the strategies of the uropathogenic *Escherichia coli* are adherence, motility, iron acquisition, toxin, and evasion of host immunity. Host immune responses play a significant part in defense of UTI. Various antimicrobial peptides (AMPs) including defensins, cathelicidin, hepcidin, ribonuclease 7, lactoferrin, lipocalin, Tamm-Horsfall protein, and secretory leukocyte proteinase inhibitor help to prevent UTI by modulation of innate and adaptive immunity. Toll-like receptors (TLRs) play an important role of microorganism identification in innate immunity. Stimulation of TLRs on the cell membrane by ligand of bacteria triggers production of inflammatory chemokines, cytokines, and AMPs. These mechanisms are an attempt to defend the urinary tract against UTI.

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections that lead to out-patient and in-patient hospital visits. About 50% of women suffer from a UTI during their lifetime [1]. About 8 million female patients are treated annually for UTI alone in the United States and UTI recurs in about 30% of those who have normal function and anatomy of urinary tract [2]. UTI comprises acute uncomplicated cystitis and acute uncomplicated pyelonephritis. Acute uncomplicated cystitis results in dysuria, frequency, or urgency in healthy and non-pregnant women [3]. The diagnosis is aided by the presence of pyuria in voided urine and positive urine culture of at least 10^3 CFU/ml [3]. Acute uncomplicated pyelonephritis results in fever, costovertebral angle tenderness and flank pain in healthy and non-pregnant women and is similar to cystitis in lower urinary tract symptoms and laboratory analyses

[4]. UTIs are commonly classified as uncomplicated and complicated. Complicated UTI occurs in patients with co-morbid illness or anatomic malformations of the urinary tract that can aggravate the risk of infection or therapeutic failure [5]. Complicating factors, for example, include the presence of catheter, obstructive uropathy, pregnancy, urinary stones, diabetes, and male gender. Most of them are main causes of pathogenesis in the host. Meanwhile, bacterial factors in UTI have been studied worldwide for decades with the main focus on *Escherichia coli* that is the most common pathogen in community acquired UTI [6]. Eighty percent to ninety percent of acute uncomplicated cystitis or acute uncomplicated pyelonephritis correspond to *E. coli* and the similar explanation applies to most complicated UTIs [7]. *E. coli* are classified into intestinal *E. coli* and extraintestinal *E. coli*. Most in vivo and clinical studies on extraintestinal *E. coli* related to UTI show that the specific adaptations of uropathogenic *E. coli* (UPEC)

cause virulence and fitness in the urinary tract [8].

UTI PATHOGENESIS: MULTIPLE VIRULENCE FACTOR

UPEC from intestine, perineum, or prepuce attack the urinary tract [9]. The virulence of UPEC is derived from specific virulence genes that are not found in commensal *E. coli* [10]. Asymptomatic bacteriuria may be infused by *E. coli* that has lost virulence genes in evolution [11]. Virulence genes encode for adhesive proteins that are expressed on the bacterial surface [10]. UPEC adhere to the mucosa through specific bacterial ligands and host cell receptors [12]. The cells activate the innate immune system and recruit inflammatory cells secreting chemokines and cytokines. Host cells inhibit UPEC by lactoferrin, Tamm-Horsfall protein (THP), immunoglobulin A (IgA), cathelicidin or defensins. UPEC can survive from host defense and invade the superficial layer of the mucosa by polysaccharide capsules, iron-sequestering molecules, or the TcpC protein that inhibit the innate immune mechanism [13]. TcpC proteins damage Toll-like receptor (TLR) and MYD88-dependent signaling pathways [13]. In addition, UPEC can impair tissues directly by secreting toxins that weaken the host cell wall. They can destroy the mucosal barrier, soft tissues, and vessels and survive by forming capsules that provide defense from defensins or host antibodies. Furthermore, they compete for nutrients by adjusting metabolic mechanisms [14]. Generally, strategies

of UPEC are adherence, motility, iron acquisition, production of toxin, and evasion of host immune (Table 1). These pathogenic mechanisms are known from several lines of research.

1. Adherence via Fimbriae

Fimbriae make UPEC adhere to host epithelial receptors. Type-1, P, F1c, Dr, Auf, S, and M fimbriae are found in *E. coli* CFT073 reference strain (Table 2) [10]. Type-1 fimbriae adhere to the transmembrane glycoproteins of bladder called uroplakins, and receptors like CD11, CD18, CD44, and CD48 [15]. Type-1 fimbriae mostly exist in UPEC, but type-1 fimbriae do not always correspond with virulence because other fecal strains also have type-1 fimbriae. Type-1 fimbriae have an adhesion molecule, FimH, which helps UPEC invade superficial cells by binding mannosylated glycoproteins [16]. P fimbriae activate host TLR4 signaling. P fimbriae are always found in strains that cause acute pyelonephritis and urosepsis [17]. P fimbriae bind to glycosphingolipid receptors and stimulate an innate immune system in the human urinary tract [18]. F1C fimbriae are detected in human UTI but pathogenicity of F1C fimbriae is unknown [19]. Dr fimbriae attach to the urothelium of bladder and type IV collagen, and are related to pathogenic cell invasion [20]. Auf fimbriae are found in UPEC but their role is not known well [21]. With Auf fimbriae, the nonfimbrial UpaG supports cell aggregation and adhesion to human urothelium. But UpaG is not essential for colonization [22]. These assorted fimbriae may help adherence to host cell avoiding immune mechanisms in different regions of the urinary tract (Table 3).

Table 1. Virulence factors of uropathogenic *Escherichia coli*

Classification	Virulence factors
Metal acquisition	heme, enterobactin, siderophores (Iron), ZnuACB (Zinc)
Toxins	Cytotoxic necrotizing factor 1, Sat, Pic, Tsh
Evasion from host defenses	Salmocheilin, SisA, SisB
Fimbriae	Type-1, P, F1c, Dr, Auf, S, M fimbriae

2. Motility via Flagella

Adherence and motility are key characteristics that have opposite properties. Motility is controlled by flagella in UPEC and this is helpful for fitness of UPEC in UTI [23]. Flagella genes are inhibited in nutrition abundant conditions

Table 2. Uropathogenic *Escherichia coli* fimbrial adhesion binding receptor

Type of fimbriae	Binding receptor
Type 1 fimbriae	Secretory IgA, UP1A, UP1b, UP2, UP3a, CD11, CD18, CD44, CD48, N-oligosaccharide on integrins β 1 and α 3, Tamm-Horsfall protein
P fimbriae	Glycosphingolipid receptor, α -D-galactopyranosyl-(1-4)- β -D-galactopyranoside receptor
Dr fimbriae	Type IV collagen, decay-accelerating factor, CD55
F1C fimbriae	Galactosylceramide target (bladder, kidney), globotriaosylceramide (kidney)
S fimbriae	Sialic acid glycolipids, glycoproteins, α -sialyl-2,3-galactose receptor

IgA: Immunoglobulin A, UP: uroplakin.

Table 3. Uropathogenic *Escherichia coli* fimbrial adhesion binding sites in kidney

Human kidney region	Type of fimbriae
Vessel walls	P, S, type 1, F1C
Glomerulus	P, S
Bowman's capsules	P, S, Dr
Proximal tubules	P, S, type 1, Dr
Distal tubules	P, S, type 1, F1C, Dr
Collecting duct	P, S, type 1, F1C, Dr

[24]. Inversely, flagella genes are upregulated in ascending UPEC infection from bladder to kidney [25].

3. Metal Acquisition

Iron is needed for survival of bacteria. *E. coli* CFT073 has more than 10 iron uptake regions [10]. Acquisition of heme, enterobactin, and siderophores supports UPEC in infection, but this is not an essential component for virulence [26]. Zinc is also a necessary nutrient. High affinity zinc transport system ZnuACB is reportedly found in UPEC [27].

4. Autotransporter Protein

Autotransporter (AT) proteins can cross the bacterial membrane and be released into the extracellular surroundings, or remain in cell surface. Thus AT proteins show various functions from cell associated adhesins to secreted toxins [28]. Antigen 43 (Ag 43) is a representative of the AT family. Ag 43 is associated with cell aggregation and biofilm formation in *E. coli* [29]. Other AT protein, UpaH also contributes to biofilm formation and bladder colonization in a mouse UTI model [30].

5. Toxins

UPEC produce α -hemolysin, cytotoxic necrotizing factor 1 (CNF1), and autotransporter toxins. α -Hemolysin is expressed by *hlyCABD* operon of UPEC [31]. α -Hemolysin invades urothelium and stimulates cytokines [32]. This toxin destroys urothelium and causes bladder hemorrhage and kidney damage [33]. CNF1 stimulates the Rho family of GTP binding proteins and supports adherence and invasion of host cells [34]. UPEC with CNF1 promotes apoptosis and more inflammation than UPEC without CNF1 [35,36]. α -Hemolysin and CNF1 were confirmed provocation of inflammation in mouse model and zebrafish infection model [37]. UPEC secretes AT toxins (Sat, Pic, and Tsh) [10]. Sat is a serine protease that damages bladder and kidney cells

[38]. Pic is also a serine protease that increases in infection, but its function is unknown [39]. Tsh lack serine protease activity, infection- induced increases, and is not essential for colonization [39].

6. Evasion from Host Defenses

Host immune system captures type 1 fimbriae via antibodies. In addition, enterobactin that is important mechanism for iron acquisition may be captured by host protein lipocalin 2 [40]. UPEC conceals type 1 fimbriae to avoid host antibodies and produces salmochelin that withstands lipocalin 2 instead of enterobactin [41]. UPEC-derived factors that interrupt cytokine and polysaccharides release also help to avoid host defenses [13]. Proteins SisA and SisB from some UPEC inhibit the host inflammatory mechanism [42]. Variation of type 1 fimbriae help UPEC to evade the host immune system.

HOST INNATE IMMUNITY AGAINST UTI

Antimicrobial peptides (AMPs) are small cationic proteins produced by white cells and epithelial cells as antibiotics, when the innate immune system is challenged by pathogens [43]. AMPs have a wide antimicrobial spectrum that includes bacteria, virus, and fungus. The antimicrobial function of AMPs is associated with electric charge, secondary structure, and amphiphilic characteristics [44]. Increased electric charge of AMPs strongly attracts the negative charge of microbial membranes. Amphipathicity is a characteristic that derives from hydrophilic and hydrophobic amino acid of AMPs that facilitates interaction with hydrophilic conditions and hydrophobic microbial membranes [45]. Secondary structure can modify antimicrobial function by 3-dimensional structure. These characteristics make AMPs stick to microorganism, block microbial binding, trigger other components of immune system, and weaken membrane of microorganism. AMPs bind to the negatively charged microbial membrane by the cationic portion, thus inhibiting membrane function and causing death of microorganisms. Some AMPs influence cellular protein or DNA synthesis by passing the cell membrane [45]. Because of these effects, AMPs are regarded as potential therapeutics for drug resistance. AMPs have some desirable properties. AMPs show antimicrobial function at low density. Because microorganisms cannot change their cell membrane easily, they maintain suscep-

Table 4. Known antimicrobial peptides (AMPs) in the human kidney and urinary tract

AMPs	
Proximal tubule	LL-37
Loop of Henle	HBD1, HBD2, HD5, LEAP-1, THP
Distal convoluted tubule	HBD1, lactoferrin, SLPI
Collecting Tubule	HBD1, HBD2, HD5, RNase 7
Renal pelvis	HD5, RNase 7
Ureter	HD5, LL-37, RNase 7
Bladder	HD5, RNase 7
Urethra	HD5

HBD: human β -defensins, HD: human α -defensin, LEAP-1: liver expressed antimicrobial peptide-1, THP: Tamm-Horsfall protein, SLPI: secretory leukocyte proteinase inhibitor, RNase 7: ribonuclease 7.

tibility to AMPs. AMPs surmount weakness of antibiotics that lose their ability to permeabilize cell membranes [46]. AMPs display synergistic effects with antibiotics [47]. Despite the broad dispersion in nature, not many AMPs are known to exist in the kidney and urinary tract. AMPs of urinary tract are defensins, cathelicidin, hepcidin, and ribonuclease 7 (RNase 7). AMPs of kidney and urinary tract are THP, lactoferrin, lipocalin and secretory leukocyte proteinase inhibitor (Table 4) [43,45].

1. Defensins

Defensins are one of the AMPs that attack bacteria, virus, fungus and protozoans [48]. Defensins not only attack foreign cells directly but also attract immature dendritic cells [45]. In humans, defensins are classed as α -defensins or β -defensins by their disulfide bridge pattern [49]. Some genes of α -defensins and β -defensins are encoded in chromosome 8p22 and 8p23, but regions of other genes display variations from 2 to 14 per diploid genome [43,50].

1) α -Defensins

α -Defensins are human neutrophil peptides (HNPs) from neutrophils [51]. α -Defensins have been found from HNP1 to HNP4 and have azurophil granules of neutrophils. Defensins promote mast cell degranulation and neutrophil chemotaxis after phagocytosis of pathogen [43,52]. In UTI, there are increased levels of HNP1, HNP2, and HNP3 in the urinary tract, and increased levels of both HNP1 and IL-8 in glomerulonephritis [53,54]. Human α -defensin 5 (HD5) are derived from mucosal epithelial cells. HD5 are found in Paneth cells of small colon and reproductive tract [55]. HD5 can attack uropathogenic bacteria and virus [56,57]. HD5 showed bactericidal activity at minimal

concentration of 0.3 μ M [58]. However, it is reported that HD5 does not reach sufficient concentrations to kill bacteria in the urinary tract [59]. The role of HD5 in innate immunity of urinary tract is not fully known.

2) β -Defensins

Human β -defensins (HBD) are found in epithelium, including the urothelium. They have direct antimicrobial action and modulate cell-mediated immunity [60]. Six human β -defensins (HBD1-6) have been found, of which, HBD1 and HBD2 are reported in the urinary tract of human. HBD1 is found in the epithelium of kidney and its peptide is continuously found in urine [61]. Although HBD1 cannot kill bacteria in the urinary tract, it blocks bacterial attachment to the urothelial membrane by covering the surface [61]. Redox of HBD1 is related to its antimicrobial action, with reduction state being more powerful than oxidation state [62]. However, redox role of HBD1 is unknown in the infected urinary tract. HBD2, HBD3, and HBD4 is expressed by proinflammatory cytokines or bacteria, unlike continuously secreted HBD1 [61,63]. HBD2 has stronger antimicrobial potency against Gram positive and negative bacteria [60]. Its antimicrobial function is attributed to lysis of the phospholipid bilayer of bacterial membrane [60,64].

2. Cathelicidin

Human cathelicidin (LL-37) is initially found in granules of neutrophils, and subsequently found in bone marrow and epithelial cells [65]. LL-37 is expressed in bacterial infection and its antimicrobial range is from bacteria to virus by chemotaxis of neutrophil and monocyte [66]. Endotoxin neutralization, angiogenesis, and wound healing are the other functions of LL-37 [67]. LL-37 is found in kidney and ureter and increases in the urinary tract in response to UPEC [68]. LL-37 is more powerful than HBD2 in the innate immune system, including the urinary tract [68,69]. LL-37 may play an important role in urinary tract defense, but its role is limited in UPEC-induced severe UTI due to bactericidal resistance [68].

3. Hepcidin

Hepcidin (liver expressed antimicrobial peptide1, LEAP1) is made in liver and secreted in the urinary tract [43]. LEAP-1 is related to iron homeostasis and overexpressed LEAP-1 results in severe iron deficiency [70]. LEAP-1 has broad

spectrum as a bactericide and its function derives from direct antimicrobial effect and reduction of usable iron that is needed for bacterial survival [71].

4. Ribonuclease 7

RNase 7 was initially found in epidermis and subsequently found in bladder, ureter and kidney [72,73]. Concentration of RNase 7 is higher than other AMPs and shows sufficient bactericidal effect. Rapid and strong action of RNase 7 from Gram positive and negative bacteria results from distraction of cell membrane, that is independent with ribonuclease activity [73]. Antimicrobial processes of RNase 7 are not fully identified.

5. Lactoferrin and Lipocalin

Lactoferrin is found in distal collecting tubules. It causes chelation of iron and modification of membrane integrity [74]. Lipocalin reduces siderophore-iron of bacteria and shows bacteriostatic function [75]. Mice without lipocalin are more susceptible to bacteria that utilized siderophores [76].

6. Tamm-Horsfall Protein

THP, the most plentiful protein in human urine, is secreted in the loop of Henle [77]. THP does not have bactericidal activity itself, but blocks bacterial binding to epithelium and promotes bacterial wash-out by urine. THP activates dendrite cells by a TLR4 dependent mechanism.

7. Secretory Leukocyte Proteinase Inhibitor

Secretory leukocyte proteinase inhibitor (SLPI) is the main protease inhibitor from kidney. SLPI has antimicrobial activity and promotes macrophages via cytokines [78]. The first NH2-terminal domain of SLPI showed bactericidal effects on *E. coli* and *Staphylococcus aureus* [79].

CURRENT AND POTENTIAL APPLICATIONS OF AMPs IN UTIs

Antibiotics are currently the main UTI treatment modality, but their drawbacks include multidrug resistance, side effects, and limitation of use such as in pregnancy or young children [80]. Several studies show that these weaknesses are overcome by AMPs. An in vivo study by Haversen et al. [80] showed that orally administered lactoferrin is

successful in the treatment of infection and inflammation of urinary tract via renal secretion. Other AMPs may reach the target site after oral intake using this mechanism. Choi et al. [81] reported that instillation of LL-37 inhibits infiltration of bacillus Calmette-Guerin in vitro. In addition, upregulation of AMPs without administration of exogenous AMPs may treat UTI. Hertting et al. [82] show that vitamin D increases LL-37 in bladder biopsies that are infected with UPEC. Rivas-Santiago et al. [83] reports that L-isoleucine upregulates HBD3 and HBD4 in mice. Furthermore, according to a study of Schwab et al. [84], butyrate upregulates LL-37 mRNA. Estrogen can also increase AMPs including HBD1-3, LL-37, and RNase 7 in the urinary tract [85]. The induction of various AMPs has important roles in mucosal defense of the urinary tract against UTI.

Catheter associated UTIs are hospital acquired UTIs that are induced by biofilms of microorganisms [86]. Minardi et al. [87] show that ureteral stents coated by Tachyplesin III, which is AMP from horseshoe crabs prevent biofilm formation in vivo. AMP coating polymer brushes with hydrophilic copolymer and poly chains on the surface that help to conjugate with AMPs, are recently introduced [88]. Polymer brush demonstrate effective antimicrobial function in vitro and in vivo [88]. Brush peptide coating catheter may likewise be useful to prevent biofilm, but more clinical studies are needed.

ROLE OF TLRs IN URINARY TRACT DEFENSE

TLRs play an important role in microorganism identification in innate immunity. TLRs are transmembrane proteins with leucine rich repeat domains that are useful to identify pathogen associated molecular patterns (Table 5). Stimulation of TLRs on the cell membrane by ligand of bacteria triggers inflammatory chemokines, cytokines, and AMPs [89]. TLR2 identifies lipoteichoic acid or lipoprotein of bacteria. Activation of TLR2 causes secretion of tumor necrosis factor (TNF) α that defend against spreading infection [90]. *Leptospira* provokes TLR2 stimulation, and monocyte chemoattractant protein 1 (MCP 1) and macrophage inflammatory protein 2 (MIP 2) are produced in renal tubule epithelial cells [91]. TLR3 identifies microbial nucleic acid and double stranded RNA; while TLR4 identifies lipopolysaccharides. TLR4 of innate immunity and bladder

Table 5. Known Toll-like receptors (TLRs) mainly expressed in urothelium

	Ligand	Organism	Location
TLR2	Lipoproteins, peptidoglycans, lipoteichoic acid, porins, lipoarabinomannan Phospholipomannans and glucuronoxyl mannans, zymosan, β -glucans HSP 70	Gram-positive bacteria, mycobacteria, mycoplasma Fungi Human	Cell surface
TLR3	Double stranded RNA	Viruses	Cell compartment
TLR4	Lipopolysaccharides Mannanes HMGB1, HSP, extracellular matrix components	Gram-negative bacteria Fungi Human	Cell surface
TLR5	Flagellin	Flagellated bacteria	Cell surface
TLR7	Single stranded RNA Self DNA/LL-37, self RNA/LL-37	RNA viruses Human	Cell compartment
TLR9	Nonmethylated CpG DNA DNA Self DNA/LL-37, self RNA/LL-37, self DNA/HMGB1	Bacteria and mycobacteria Viruses, parasites Human	Cell compartment
TLR11 (in mice)	Profilin	Toxoplasma gondii, uropathogenic bacteria	Cell compartment

HSP: heat shock protein, HMGB1: high-mobility group protein B1.

urothelium plays important roles in UTI [92]. Mice without TLR4 cannot prevent cell surface from removing UPEC and cannot secrete proinflammatory cytokines and chemokines. TLR5 identifies bacterial flagellin. Uropathogenic bacteria with flagella has easier access to the upper urinary tract [25]. Mice without TLR5 are more susceptible to UPEC [93]. TLR7 identifies single stranded RNA of virus. Administration of TLR7 agonist in bladder increases TNF α and chemokine [94]. TLR9 identifies unmethylated DNA of bacteria and viruses. TLR11 identifies profilin of Toxoplasma that can infect renal cells [95]. Mice without TLR11 are more susceptible to UPEC in kidney [96]. In vivo mice studies show that among these TLRs of urothelium, only TLR4, TLR5, and TLR11 play a protective role in UTI [89,97]. TLR4 is found in kidney and bladder cells; while TLR5 is mainly found in bladder cells and TLR11 mostly in kidney cells. However, TLR11 is detected in mice, but is not yet found in humans.

ADAPTIVE IMMUNITY IN THE URINARY TRACT

Bacteria can still penetrate the innate immune defense, so defense of the urinary tract further involves adaptive immunity. Both cellular and humoral immunity are increased after UPEC infection. Activated T cells migrate to bladder and specific IgG antibodies are secreted in the serum and urine [98]. In addition, sensitized B lymphocytes are recruited in lamina propria and secretory IgA are increased.

These antibodies prevent bacteria from colonizing by blocking bacterial adhesion or helping opsonization by phagocyte [99]. Adaptive immunity also plays a significant role in protection from UTI.

CONCLUSIONS

Mucus secretion and covering of AMPs in response to bacterial penetration of mechanical barriers of urine flow facilitates the spread of infection to the upper urinary tract. UPEC stimulates TLRs that trigger inflammatory chemokines, cytokines, and AMPs. Leukocytes move to the infection site following chemokine release, causing further increases in secreted AMPs. Adaptive immunity also has a role in protection from UTI. These mechanisms reflect an attempt to defend the urinary tract against UTI.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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