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Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection

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Abstract

Urinary tract infections (UTI) are among the most common infectious diseases of humans and are the most common nosocomial infection in the developed world. It is estimated that 40-50% of women and 5% of men will develop a UTI in their lifetime, and UTI accounts for more than 1 million hospitalizations and \$1.6 billion in medical expenses each year in the USA. Uropathogenic *Escherichia coli* (UPEC) is the primary cause of UTI. This review presents an overview of recent discoveries related to the primary virulence factors of UPEC and major innate immune responses to infection of the lower urinary tract. New and emerging themes in UPEC research are discussed in the context of the interface between host and pathogen.

Introduction

Urinary tract infections (UTI) are one of the most common bacterial infections of humans and are a major cause of morbidity. UTI usually starts as a bladder infection (cystitis), but can develop to acute kidney infection (pyelonephritis), ultimately resulting in scarring and renal failure. UTI is caused by a range of pathogens, with uropathogenic *Escherichia coli* (UPEC) being the most common etiological agent. This review will focus on UPEC, discussing recent advances in our knowledge of its virulence factors and innate immune responses to acute bladder infection.

UPEC express multiple virulence factors that promote UTI

UPEC cause more than 80% of all UTI. UPEC strains possess an arsenal of virulence factors that contribute to their ability to cause disease, including fimbrial adhesins, toxins, flagella, autotransporter proteins and iron-acquisition systems [1]. UPEC fitness in the nutritionally poor urinary tract is also aided by the utilization of short peptides and amino acids as a carbon source during infection [2] as well as the presence of type II toxin-antitoxin systems [3].

UPEC adherence to the urinary tract epithelium is primarily mediated by fimbriae assembled by the chaperone-usher pathway [4] (Box 1). Type 1 fimbriae, one of the best-characterized UPEC chaperone-usher fimbriae, bind to α -D-mannosylated proteins such as uroplakins that are abundant in the bladder via the tip-located FimH adhesin. Type 1 fimbriae enhance colonization and activation of host innate immune pathways in the murine UTI model, and promote biofilm formation and host cell invasion [1]. FimH-mediated binding to target receptors is enhanced through the formation of catch bonds, mechanical forces that contribute to FimH-receptor complex interactions [5]. Recent work

has also shown that FimH is recognized by host pattern recognition receptors (PRRs), thus leading to potent induction of innate antimicrobial responses [6]. Additional UPEC surface components that contribute to colonization of the urinary tract include established factors such as other fimbriae (e.g. P, F1C, S and Afa) [7] and more recently identified factors such as curli [8], autotransporter proteins (e.g. Ag43, UpaH) [9,10], TosA [11] and flagella [12].

UPEC secrete a number of toxins that damage or kill host epithelial cells. One of the most common UPEC toxins, α -hemolysin, mediates host cell lysis, thus promoting the release of nutrients such as iron that can be utilized by UPEC for growth and/or survival. Sublytic concentrations of α -hemolysin also contribute to virulence by enabling UPEC to modulate epithelial cell functions as will be discussed later [13]. Another toxin, cytotoxic necrotizing factor 1 (CNF1) is a Rho GTPase that promotes invasion of UPEC into host cells [14]. Recent data suggest that UPEC CNF1 and α -hemolysin may contribute to the signs and symptoms of cystitis (along with LPS as discussed further below) [15]. In the zebrafish model, α -hemolysin and CNF1 function primarily in the neutralization of phagocytes [16].

Iron is essential for bacterial growth and is limited in the urinary tract. Four different Fe^{3+} -chelating siderophore systems have been characterized in UPEC, namely enterobactin, the glucosylated enterobactin derivative salmochelin, yersiniabactin and aerobactin [17]. UPEC strains may express different combinations of these siderophores, with some strains able to express all four siderophores [18]. The siderophore repertoire expressed by a given UPEC strain may influence the ability of the bacteria to grow and persist in human urine [19]. Siderophores also possess functions distinct from iron binding; for example, Chaturvedi *et al.* showed that yersiniabactin can sequester host-derived copper (II), thus

enhancing resistance to copper stress [20]. Many UPEC strains also express heme receptors (e.g. ChuA and Hma) that enable iron uptake and contribute to virulence [21]. An increased understanding of the role of iron acquisition systems, and indeed additional systems required for the transport of other transition metals such as zinc, copper and manganese, may uncover new concepts in UPEC virulence and nutritional immunity at the host-pathogen interface [22].

Intracellular and extracellular lifestyles are hallmarks of UPEC

UPEC pathogenesis during experimental UTI involves the occupancy of both extracellular and intracellular niches. Prototype UPEC strains including the pyelonephritis strain CFT073 and the cystitis strain UTI89 possess different sets of virulence factors and utilize these lifestyles to different degrees. For example, CFT073 is a highly toxigenic strain that can cause severe damage to the urothelium and immunopathology [23], but can also invade epithelial cells and form intracellular bacterial communities (IBCs) [24]. In contrast, UTI89 is a more invasive strain that forms IBCs and survives intracellularly, but also expresses several toxins that cause urothelial damage [25]. The mosaic nature of the UPEC genome means that there are no unique genetic features that clearly distinguish these different lifestyles. Recent attempts to define UPEC population dynamics during infection may also reflect differences between these two broad host-adapted lifestyle traits [26,27]. Added to the complexity of these different UPEC lifestyles is the emergence of multidrug resistant globally disseminated clones such as *E. coli* of serotype O25b:H4 and sequence type 131 (*E. coli* ST131), for which a genome sequence and particular key virulence mechanisms were recently described [28]. For cystitis strains like UTI89, type 1 fimbriae-mediated adherence to superficial bladder facet cells leads to invasion, rapid bacterial replication in the facet cell cytoplasm and the formation of IBCs [29]. These

events are dependent on the FimH adhesin and are associated with specific amino acid residues that are under positive selection [30]. Other UPEC factors that contribute to IBC formation include Ag43, the polysaccharide capsule and sialic acid [31]. IBC formation culminates in the bursting of superficial facet cells and the release of UPEC, often as long filamentous bacteria [32]. IBCs may enable UPEC evasion of the host immune response, permit re-infection and contribute to chronicity [29]. IBCs and filamentous bacteria have also been observed in urine from women suffering acute cystitis [33]. A comprehensive inventory of UPEC biofilm-associated genes was recently mapped using transposon mutagenesis and may provide a framework for further analysis of UPEC extracellular and intracellular biofilm growth [34]. UPEC can also establish quiescent intracellular reservoirs (QIRs) that contain small numbers of bacteria and may play a role in latent chronic infection and recurrent UTI [35]. The ability of UPEC to survive intracellularly is not limited to epithelial cells. Some UPEC strains can survive in primary mouse macrophages within lysosomal-associated membrane protein 1-positive vesicles, a property that may contribute to their dissemination in the urinary tract [36].

Innate immune responses to UPEC control but may also predispose to UTI

Several findings over the past few years continue to inform the view that UPEC cystitis is not a simple condition that develops, is detected and resolved by management, and leaves a convalescent host without further implications for disease. Increased risk for recurrent UTI subsequent to primary cystitis has been known for sometime, but recent studies have uncovered new contributions of innate defenses to pain, symptoms, defense, and predisposition to chronicity (Figure 1). Hannan *et al.* showed that inflammatory events activated in the bladder during early responses to UPEC in mice provide the immunological stage for subsequent chronicity and susceptibility to recurrent infection [37].

Signature responses comprising interleukin (IL)-5, IL-6, and granulocyte-colony stimulating factor (G-CSF) appear to mediate increased susceptibility in a Toll-like Receptor (TLR)4-dependent manner, which suggests involvement of UPEC lipopolysaccharide (LPS) and/or P fimbriae [38]. Two genome-wide transcriptomic studies by Duell *et al.* and Tan *et al.* have provided insight into the factors that might contribute to innate resistance and/or susceptibility to UPEC on a global scale [39,40]. These studies build on prior analyses of mice with cystitis [41] and show that bladder inflammation in response to UPEC is rapid, pathogen-specific, and extensive encompassing 1564-2507 active genes that drive diverse canonical pathways such as IL-10, IL-17A, TLR, and NOD-like receptor signalling, as well as networks for cell movement, death, proliferation and maturation [39,40]. Collectively, this suggests that UPEC may somehow harness complex innate immune responses in the bladder to promote bacterial survival, predisposition to UTI and chronicity.

The double-edge sword of innate immune responses to UPEC does not appear to be limited to experimental models; a recent description of patients that progressed from cystitis to UPEC bacteremia, for example, suggests clinical relevance. In individuals with cystitis, Marschall *et al.* showed that specific symptoms of hesitancy/retention are a risk factor for progression to urinary-source bacteremia [42]. UTI symptoms such as pelvic pain appear to have a basis in local inflammatory events such as TLR signalling [43], possibly linking innate immune activation, pain and UTI progression. However, the O-antigen of UPEC LPS appears to play a role in the pain sensation independent of inflammation [44]. Also in the murine model of UTI, pain occurs independent of the level of bacterial colonization and inflammation, and pain can persist after clearance of UPEC from the genitourinary tissues [45]. While toxins contribute to inflammation and especially exfoliation and/or destruction of the urothelium toxin expression is not required for

inflammation in acute UTI and the contribution of toxins to pain responses have yet to be elucidated [15]. Thus, while recent data hint at potential links between inflammatory events and pain, studies are now needed to define which inflammatory pathways and molecules contribute to pain, how host genetic background impacts on these responses, and the extent to which these pathways are triggered in patients during acute versus subacute UTI. Clinically relevant models such as described in [37] will be essential to further define the basis of pain, severity and progression in experimental UTI. TLR4 signalling [43] has also been associated with inflammation and acute UTI because TLR4-deficient mice develop asymptomatic infection [46], although questions about other unidentified genes and/or PRRs that may impact host susceptibility (Box 2) warrant further investigation [47].

Recently identified innate mechanisms that constrain UPEC: peptides, receptors, and cytokines

Recent discoveries on the antimicrobial peptide cathelicidin, as well as the erythropoietin and P2Y receptors, have revealed new aspects of defense against UPEC. Production of cathelicidin constrains UPEC in the bladder, and its production is boosted by vitamin D, which may represent a potential new adjunct for the prevention of UTI [48]. New insight into potential therapeutic avenues is also provided from discoveries on invasion into urothelial cells. Polgarova *et al.* described a synthetic erythropoietin analogue that modifies early steps in the host response to UPEC by moderating IL-8 production and reducing UPEC invasion [49]. This could aid in the elimination of bacteria while reducing immunopathology that has been linked to chronic and recurrent infection [37]. Extracellular ATP and P2Y receptor activation appear to drive IL-8 production [50], representing an alternate mechanism of non-TLR4-driven pro-inflammatory cytokine production in urothelial cells infected with UPEC. Finally, Erman *et al.* demonstrated that UPEC induces

serum amyloid A, an acute phase protein, and that this constrained early UPEC colonization of the bladder [51]. This effect may stem from prevention of biofilm formation, however other mechanisms may also be involved; for example, serum amyloid A promotes IL-10 production from neutrophils [52], which could contribute to beneficial effects.

Two recent discoveries on IL-17 [53] and IL-10 [39] have revealed protective roles in UPEC infection. Both cytokines, like G-CSF [54], are produced following UPEC infection, but unlike G-CSF, they appear to somehow restrict UPEC's ability to maintain bladder infection. Ingersoll *et al.* showed that neutrophils are rapidly recruited to the mouse bladder in a G-CSF-dependent manner and reported an association between increased UPEC survival and reduced neutrophil responses [54]. Others previously showed that neutrophil responses in the infected urinary tract depend on host genetic background and are reliant on macrophage inflammatory protein-2 [55,56]. The mechanisms by which reduced G-CSF responses directly promote UPEC survival remain unknown, as discussed elsewhere [54]. The known actions of IL-17 and IL-10 towards cell recruitment and immune regulation [57,58] imply that these cytokines may be required to fine tune innate cellular defenses to UPEC in the bladder. Recent insights into NF-kappaB [59] and IRF3-dependent signalling [60] also point to activation of specific signalling pathways to distinct *E. coli* strains that trigger different mechanisms of innate immune activation [40,61]. Thus, further analysis of IL-17, IL-10, and G-CSF regulation and function will be needed to specify their role in UTI considering these new insights.

UPEC Employ Multiple Mechanisms to Curb Innate Immune Responses

Manipulation of innate immune responses by UPEC may enhance their survival [62] and there are several recent examples of this. Some UPEC strains secrete TcpC, a Toll/IL-1 receptor (TIR) domain-containing protein that exhibits structural similarity to the TIR domain of human TLR1 [63]. TcpC inhibits TIR domain signalling and downstream pathways through myeloid differentiation primary response protein-dependent and -independent effects [64]. Wang *et al.* has shown that expression of dynamin2- and endothelial nitric oxide synthase is driven by UPEC and promotes invasion into host cells [65]. Two other recent demonstrations by Hilbert *et al.* and Dhakal *et al.* show that UPEC utilizes α -hemolysin to inhibit epithelial cytokine production, as well as cell adhesion and inflammation [13,66]. Finally, UPEC curli were recently shown to interact with the antimicrobial peptide cathelicidin LL-37, and thereby modulate early inflammatory events mounted against infection [8].

Autophagy has emerged as an important mechanism in bacterial pathogenesis, and recent data suggests that this process is involved in innate defense against UPEC. Wang *et al.* demonstrated a pro-pathogen role for the autophagy protein Atg16L1; deficiency in this factor conferred protection against infection [67]. Utilization of autophagy pathways by UPEC may impede the release of inflammatory cytokines such as IL-1 β [68]. Two other separate studies on attenuation of innate responses by UPEC in the context of dampening innate responses offer alternative avenues for further investigation; firstly, the discovery that cyclooxygenase-2 is regulated by UPEC in urothelial cells [69], and secondly, the finding that indoleamine 2,3-dioxygenase is induced by UPEC, which attenuates innate responses to epithelial infection [70]. Jointly, these data suggest a potential link between cyclooxygenase-2 and indoleamine 2,3-dioxygenase in UPEC mediated UTI, as reviewed

elsewhere in the context of non-infectious diseases [71].

Challenges, Opportunities and Future Research Directions

As with many pathogens, UPEC employs multiple strategies to evade and manipulate host barrier defence and innate immune responses. Our increased understanding of these pathogen-host interactions has uncovered novel approaches that could be used to combat UPEC mediated UTI, such as strategies aimed at selectively boosting the production or function of molecules like IL-10, IL-17, cathelicidin and serum amyloid A. Novel therapeutics using pathogen-derived molecules that directly impact innate immune responses and manipulate host response pathways also seem plausible. These areas of investigation should be considered in view of the genotypic and phenotypic diversity of UPEC clonal groups, and the wide spectrum of UTI pathologies associated with different strains. While research has focused on prototype UPEC strains such as CFT073 and UTI89, future research also needs to study emerging strains. In particular, the pathogenesis mechanisms employed by multidrug resistant UPEC strains such as the globally disseminated *E. coli* ST131 clone should be addressed.

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References

1. Nielubowicz GR, Mobley HL: **Host-pathogen interactions in urinary tract infection.** *Nature reviews. Urology* 2010, **7**:430-441.
2. Alteri CJ, Smith SN, Mobley HL: **Fitness of *Escherichia coli* during urinary tract infection requires gluconeogenesis and the TCA cycle.** *PLoS Pathog* 2009, **5**:e1000448.
3. Norton JP, Mulvey MA: **Toxin-antitoxin systems are important for niche-specific colonization and stress resistance of uropathogenic *Escherichia coli*.** *PLoS Pathog* 2012, **8**:e1002954.
4. Busch A, Waksman G: **Chaperone-usher pathways: diversity and pilus assembly mechanism.** *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 2012, **367**:1112-1122.
5. Sokurenko EV, Vogel V, Thomas WE: **Catch-bond mechanism of force-enhanced adhesion: counterintuitive, elusive, but ... widespread?** *Cell Host Microbe* 2008, **4**:314-323.
6. Ashkar AA, Mossman KL, Coombes BK, Gyles CL, Mackenzie R: **FimH adhesin of type 1 fimbriae is a potent inducer of innate antimicrobial responses which requires TLR4 and type 1 interferon signalling.** *PLoS Pathog* 2008, **4**:e1000233.
7. Korea CG, Ghigo JM, Beloin C: **The sweet connection: Solving the riddle of multiple sugar-binding fimbrial adhesins in *Escherichia coli*: Multiple *E. coli* fimbriae form a versatile arsenal of sugar-binding lectins potentially involved in surface-colonisation and tissue tropism.** *Bioessays* 2011, **33**:300-311.
- * 8. Kai-Larsen Y, Luthje P, Chromek M, Peters V, Wang X, Holm A, Kadas L, Hedlund KO, Johansson J, Chapman MR, *et al.*: **Uropathogenic *Escherichia coli***

modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. *PLoS Pathog* 2010, **6**:e1001010.

- * Curli and cellulose, factors that mediate biofilm formation by UPEC, were examined for their ability to provide protection against innate immune defense mechanisms of the urinary tract. Curli fibres reduced UPEC sensitivity to the human antimicrobial peptide LL-37, while cellulose reduced immune induction during infection.
9. Allsopp LP, Totsika M, Tree JJ, Ulett GC, Mabbett AN, Wells TJ, Kobe B, Beatson SA, Schembri MA: **UpaH is a newly identified autotransporter protein that contributes to biofilm formation and bladder colonization by uropathogenic *Escherichia coli* CFT073.** *Infect Immun* 2010, **78**:1659-1669.
10. Ulett GC, Valle J, Beloin C, Sherlock O, Ghigo JM, Schembri MA: **Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract.** *Infect Immun* 2007, **75**:3233-3244.
11. Vigil PD, Wiles TJ, Engstrom MD, Prasov L, Mulvey MA, Mobley HL: **The repeat-intoxin family member TosA mediates adherence of uropathogenic *Escherichia coli* and survival during bacteremia.** *Infect Immun* 2012, **80**:493-505.
12. Lane MC, Alteri CJ, Smith SN, Mobley HL: **Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract.** *Proc Natl Acad Sci U S A* 2007, **104**:16669-16674.
- ** 13. Dhakal BK, Mulvey MA: **The UPEC pore-forming toxin alpha-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways.** *Cell Host Microbe* 2012, **11**:58-69.
- ** The authors demonstrate that the UPEC toxin α -hemolysin can stably insert into epithelial cell and macrophage membranes, resulting in the modulation of a range

of epithelial cell functions as well as the suppression of macrophage inflammatory responses.

14. Dhakal BK, Kulesus RR, Mulvey MA: **Mechanisms and consequences of bladder cell invasion by uropathogenic *Escherichia coli***. *Eur J Clin Invest* 2008, **38 Suppl 2**:2-11.
15. Smith YC, Rasmussen SB, Grande KK, Conran RM, O'Brien AD: **Hemolysin of uropathogenic *Escherichia coli* evokes extensive shedding of the uroepithelium and hemorrhage in bladder tissue within the first 24 hours after intraurethral inoculation of mice**. *Infect Immun* 2008, **76**:2978-2990.
16. Wiles TJ, Bower JM, Redd MJ, Mulvey MA: **Use of zebrafish to probe the divergent virulence potentials and toxin requirements of extraintestinal pathogenic *Escherichia coli***. *PLoS Pathog* 2009, **5**:e1000697.
17. Henderson JP, Crowley JR, Pinkner JS, Walker JN, Tsukayama P, Stamm WE, Hooton TM, Hultgren SJ: **Quantitative metabolomics reveals an epigenetic blueprint for iron acquisition in uropathogenic *Escherichia coli***. *PLoS Pathog* 2009, **5**:e1000305.
- * 18. Watts RE, Totsika M, Challinor VL, Mabbett AN, Ulett GC, De Voss JJ, Schembri MA: **Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria *Escherichia coli***. *Infect Immun* 2012, **80**:333-344.
- * The asymptomatic bacteriuria strain *E. coli* 83972 is used to determine the contribution of enterobactin, salmochelin, aerobactin and yersiniabactin to iron acquisition, growth, fitness and colonisation of the urinary tract. In addition, a role for the FecA ferric citrate receptor in iron uptake during growth in iron-limited pooled human urine is demonstrated.

19. Roos V, Ulett GC, Schembri MA, Klemm P: **The asymptomatic bacteriuria *Escherichia coli* strain 83972 outcompetes uropathogenic *E. coli* strains in human urine.** *Infect Immun* 2006, **74**:615-624.
- ** 20. Chaturvedi KS, Hung CS, Crowley JR, Stapleton AE, Henderson JP: **The siderophore yersiniabactin binds copper to protect pathogens during infection.** *Nat Chem Biol* 2012.
- ** A novel virulence function is described for the UPEC siderophore yersiniabactin. The authors demonstrate that yersiniabactin contributes to the ability of UPEC to resist copper toxicity by sequestering host-derived copper (II).
- * 21. Garcia EC, Brumbaugh AR, Mobley HL: **Redundancy and specificity of *Escherichia coli* iron acquisition systems during urinary tract infection.** *Infect Immun* 2011, **79**:1225-1235.
- * The authors assess the relative contribution of specific UPEC iron acquisition systems in UTI by constructing a series of receptor mutants in the UPEC strains CFT073 and 536, and then testing their ability to colonise the urinary tract in mixed competition experiments.
22. Hood MI, Skaar EP: **Nutritional immunity: transition metals at the pathogen-host interface.** *Nature Reviews. Microbiology* 2012, **10**:525-537.
23. Mobley HL, Green DM, Trifillis AL, Johnson DE, Chippendale GR, Lockett CV, Jones BD, Warren JW: **Pyelonephritogenic *Escherichia coli* and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains.** *Infect Immun* 1990, **58**:1281-1289.
24. Garofalo CK, Hooton TM, Martin SM, Stamm WE, Palermo JJ, Gordon JI, Hultgren SJ: ***Escherichia coli* from urine of female patients with urinary tract infections is**

competent for intracellular bacterial community formation. *Infect Immun* 2007, **75**:52-60.

25. Chen SL, Hung CS, Xu J, Reigstad CS, Magrini V, Sabo A, Blasiar D, Bieri T, Meyer RR, Ozersky P, *et al.*: **Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach.** *Proc Natl Acad Sci U S A* 2006, **103**:5977-5982.

* 26. Schwartz DJ, Chen SL, Hultgren SJ, Seed PC: **Population dynamics and niche distribution of uropathogenic *Escherichia coli* during acute and chronic urinary tract infection.** *Infect Immun* 2011, **79**:4250-4259.

* The authors construct a panel of 40 unique genetically tagged UPEC clones based on the cystitis strain UTI89 and use this to study population flux dynamics during infection of the mouse bladder.

* 27. Walters MS, Lane MC, Vigil PD, Smith SN, Walk ST, Mobley HL: **Kinetics of uropathogenic *Escherichia coli* metapopulation movement during urinary tract infection.** *mBio* 2012, **3**.

* Similar study to that described by Schwartz *et al.*, except using the pyelonephritis strain CFT073 and employing qPCR to measure the relative abundance of each tagged strain during UTI.

** 28. Totsika M, Beatson SA, Sarkar S, Phan MD, Petty NK, Bachmann N, Szubert M, Sidjabat HE, Paterson DL, Upton M, *et al.*: **Insights into a multidrug resistant *Escherichia coli* pathogen of the globally disseminated ST131 lineage: genome analysis and virulence mechanisms.** *PLoS One* 2011, **6**:e26578.

** The authors determine the draft genome sequence of a multidrug resistant *E. coli* ST131 strain. A transposon insertion in the *fimB* gene encoding the activator of type 1 fimbriae is identified and shown to be common among *E. coli* ST131 isolates from

different geographic origins. The expression of type 1 fimbriae could still be induced in fimB null strains, and this correlated with colonisation of the mouse bladder.

29. Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA, Hultgren SJ: **Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection.** *FEMS Microbiol Rev* 2012, **36**:616-648.
30. Chen SL, Hung CS, Pinkner JS, Walker JN, Cusumano CK, Li Z, Bouckaert J, Gordon JI, Hultgren SJ: **Positive selection identifies an *in vivo* role for FimH during urinary tract infection in addition to mannose binding.** *Proc Natl Acad Sci U S A* 2009, **106**:22439-22444.
31. Anderson GG, Goller CC, Justice S, Hultgren SJ, Seed PC: **Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis.** *Infect Immun* 2010, **78**:963-975.
32. Justice SS, Hunstad DA, Seed PC, Hultgren SJ: **Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection.** *Proc Natl Acad Sci U S A* 2006, **103**:19884-19889.
33. Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ: **Detection of intracellular bacterial communities in human urinary tract infection.** *PLoS Med* 2007, **4**:e329.
34. Hadjifrangiskou M, Gu AP, Pinkner JS, Kostakioti M, Zhang EW, Greene SE, Hultgren SJ: **Transposon Mutagenesis Identifies Uropathogenic *Escherichia coli* Biofilm Factors.** *J Bacteriol* 2012, **194**:6195-6205.
35. Mysorekar IU, Hultgren SJ: **Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract.** *Proc Natl Acad Sci U S A* 2006, **103**:14170-14175.

36. Bokil NJ, Totsika M, Carey AJ, Stacey KJ, Hancock V, Saunders BM, Ravasi T, Ulett GC, Schembri MA, Sweet MJ: **Intramacrophage survival of uropathogenic *Escherichia coli*: differences between diverse clinical isolates and between mouse and human macrophages.** *Immunobiology* 2011, **216**:1164-1171.
- ** 37. Hannan TJ, Mysorekar IU, Hung CS, Isaacson-Schmid ML, Hultgren SJ: **Early severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection.** *PLoS Pathog* 2010, **6**.
- ** Using an experimental mouse model, the authors demonstrate that chronic UPEC infection of the bladder is preceded by acute inflammatory responses including severe pyuria, bladder inflammation with mucosal injury, and a distinct serum cytokine signature. Mice deficient in TLR4 signaling, which are unable to generate these acute inflammatory responses, fail to develop chronic UTI. Chronic UTI that is cleared by antibiotic treatment is also shown to increase susceptibility to further UTI.
38. Frendeus B, Wachtler C, Hedlund M, Fischer H, Samuelsson P, Svensson M, Svanborg C: ***Escherichia coli* P fimbriae utilize the Toll-like receptor 4 pathway for cell activation.** *Mol Microbiol* 2001, **40**:37-51.
- ** 39. Duell BL, Carey AJ, Tan CK, Cui X, Webb RI, Totsika M, Schembri MA, Derrington P, Irving-Rodgers H, Brooks AJ, *et al.*: **Innate Transcriptional Networks Activated in Bladder in Response to Uropathogenic *Escherichia coli* Drive Diverse Biological Pathways and Rapid Synthesis of IL-10 for Defense against Bacterial Urinary Tract Infection.** *J Immunol* 2012, **188**:781-792.
- ** This study defines the first whole bladder transcriptome in mice infected with UPEC based on genome-wide transcriptional profiling. Canonical pathways that are triggered by UPEC including IL-10 signalling were used to describe a phenotype of

exacerbation of cystitis in IL-10-deficient mice, and elevated urinary IL-10 in patients with cystitis, implying a role for IL-10 in innate responses to UPEC UTI.

- * 40. Tan CK, Carey AJ, Cui X, Webb RI, Ipe D, Crowley M, Cripps AW, Benjamin WH, Jr., Ulett KB, Schembri MA, *et al.*: **Genome-Wide Mapping of Cystitis Due to *Streptococcus agalactiae* and *Escherichia coli* in Mice Identifies a Unique Bladder Transcriptome That Signifies Pathogen-Specific Antimicrobial Defense against Urinary Tract Infection.** *Infect Immun* 2012, **80**:3145-3160.
- * Comparative gene expression profiling of the bladder in UPEC-infected and streptococci-infected mice defined a 15-fold larger collection of genes with altered expression due to UPEC. This defines the pathogen-specific and conserved antimicrobial pathways in different settings of bacterial UTI, and emphasizes the unique nature of bladder innate immune activation mechanisms triggered by UPEC.
- 41. Mysorekar IU, Mulvey MA, Hultgren SJ, Gordon JI: **Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic *Escherichia coli*.** *J Biol Chem* 2002, **277**:7412-7419.
- 42. Marschall J, Zhang L, Foxman B, Warren DK, Henderson JP: **Both host and pathogen factors predispose to *Escherichia coli* urinary-source bacteremia in hospitalized patients.** *Clin Infect Dis* 2012, **54**:1692-1698.
- * 43. Rudick CN, Billips BK, Pavlov VI, Yaggie RE, Schaeffer AJ, Klumpp DJ: **Host-pathogen interactions mediating pain of urinary tract infection.** *J Infect Dis* 2010, **201**:1240-1249.
- * A study of the molecular mechanisms underlying UTI-induced pain in the murine model. The data suggest that UPEC-induced pain is independent of mast cells, UPEC colonization and neutrophils, and is not influenced by the expression of type 1

fimbriae. The authors propose a novel mechanism of infection-associated pain that is dependent on activation of TLR4, yet independent of inflammation.

44. Rudick CN, Jiang M, Yaggie RE, Pavlov VI, Done J, Heckman CJ, Whitfield C, Schaeffer AJ, Klumpp DJ: **O-antigen modulates infection-induced pain states.** *PLoS One* 2012, **7**:e41273.
45. Rudick CN, Berry RE, Johnson JR, Johnston B, Klumpp DJ, Schaeffer AJ, Thumbikat P: **Uropathogenic *Escherichia coli* induces chronic pelvic pain.** *Infect Immun* 2011, **79**:628-635.
46. Ragnarsdottir B, Lutay N, Gronberg-Hernandez J, Koves B, Svanborg C: **Genetics of innate immunity and UTI susceptibility.** *Nature reviews. Urology* 2011, **8**:449-468.
47. Suhs KA, Marthaler BR, Welch RA, Hopkins WJ: **Lack of association between the Tlr4 (Lpsd/Lpsd) genotype and increased susceptibility to *Escherichia coli* bladder infections in female C3H/HeJ mice.** *mBio* 2011, **2**:e00094-00011.
48. Hertting O, Holm A, Luthje P, Brauner H, Dyrdak R, Jonasson AF, Wiklund P, Chromek M, Brauner A: **Vitamin D induction of the human antimicrobial Peptide cathelicidin in the urinary bladder.** *PLoS One* 2010, **5**:e15580.
49. Polgarova K, Luthje P, Cerami A, Brauner A: **The erythropoietin analogue ARA290 modulates the innate immune response and reduces *Escherichia coli* invasion into urothelial cells.** *FEMS Immunol Med Microbiol* 2011, **62**:190-196.
50. Save S, Persson K: **Extracellular ATP and P2Y receptor activation induce a proinflammatory host response in the human urinary tract.** *Infect Immun* 2010, **78**:3609-3615.
51. Erman A, Lakota K, Mrak-Poljsak K, Blango MG, Krizan-Hergouth V, Mulvey MA, Sodin-Semrl S, Veranic P: **Uropathogenic *Escherichia coli* induces serum**

amyloid a in mice following urinary tract and systemic inoculation. *PLoS One* 2012, **7**:e32933.

52. De Santo C, Arscott R, Booth S, Karydis I, Jones M, Asher R, Salio M, Middleton M, Cerundolo V: **Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A.** *Nat Immunol* 2010, **11**:1039-1046.
- ** 53. Sivick KE, Schaller MA, Smith SN, Mobley HL: **The innate immune response to uropathogenic *Escherichia coli* involves IL-17A in a murine model of urinary tract infection.** *J Immunol* 2010, **184**:2065-2075.
- ** A study of IL-17A in the murine UTI model. While IL-17A is dispensable for the generation of a protective response to UPEC, its importance in innate immunity is demonstrated by a defect in acute clearance of UPEC in IL-17A-deficient mice. This may be related to an impaired inflammatory infiltrate during infection, and proves IL-17A is a key mediator of innate immune response to UTIs.
54. Ingersoll MA, Kline KA, Nielsen HV, Hultgren SJ: **G-CSF induction early in uropathogenic *Escherichia coli* infection of the urinary tract modulates host immunity.** *Cell Microbiol* 2008, **10**:2568-2578.
55. Haraoka M, Hang L, Frendeus B, Godaly G, Burdick M, Strieter R, Svanborg C: **Neutrophil recruitment and resistance to urinary tract infection.** *J Infect Dis* 1999, **180**:1220-1229.
56. Hang L, Haraoka M, Agace WW, Leffler H, Burdick M, Strieter R, Svanborg C: **Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract.** *J Immunol* 1999, **162**:3037-3044.

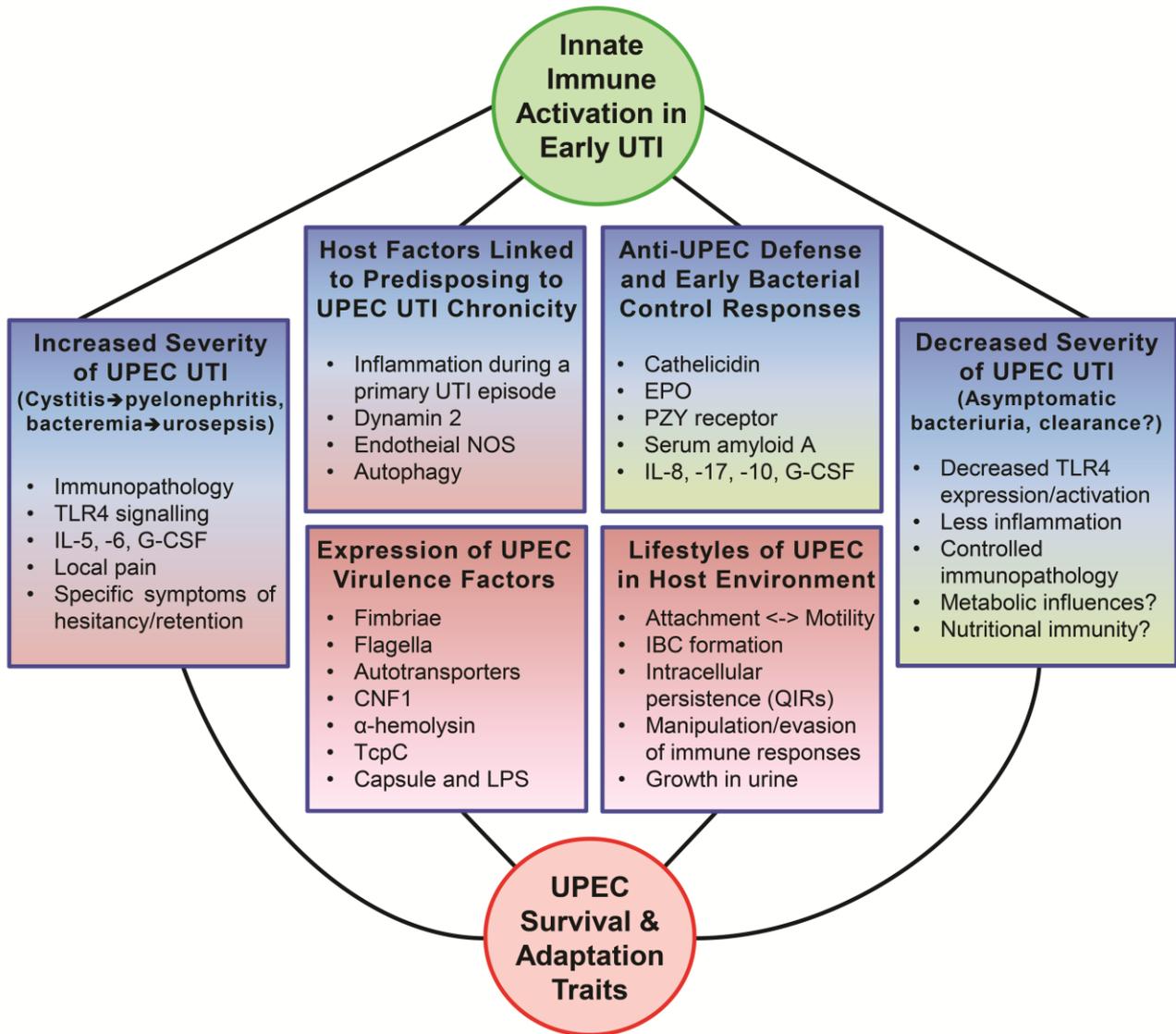
57. Duell BL, Tan CK, Carey AJ, Wu F, Cripps AW, Ulett GC: **Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis.** *FEMS Immunol Med Microbiol* 2012, **64**:295-313.
58. Rubino SJ, Geddes K, Girardin SE: **Innate IL-17 and IL-22 responses to enteric bacterial pathogens.** *Trends Immunol* 2012, **33**:112-118.
59. Karlsson M, Scherbak N, Reid G, Jass J: **Lactobacillus rhamnosus GR-1 enhances NF-kappaB activation in Escherichia coli-stimulated urinary bladder cells through TLR4.** *BMC Microbiol* 2012, **12**:15.
60. Fischer H, Lutay N, Ragnarsdottir B, Yadav M, Jonsson K, Urbano A, Al Hadad A, Ramisch S, Storm P, Dobrindt U, *et al.*: **Pathogen specific, IRF3-dependent signaling and innate resistance to human kidney infection.** *PLoS Pathog* 2010, **6**:e1001109.
61. Ulett GC, Webb RI, Ulett KB, Cui X, Benjamin WH, Crowley M, Schembri MA: **Group B Streptococcus (GBS) urinary tract infection involves binding of GBS to bladder uroepithelium and potent but GBS-specific induction of interleukin 1alpha.** *J Infect Dis* 2010, **201**:866-870.
62. Billips BK, Schaeffer AJ, Klumpp DJ: **Molecular basis of uropathogenic *Escherichia coli* evasion of the innate immune response in the bladder.** *Infect Immun* 2008, **76**:3891-3900.
63. Cirl C, Wieser A, Yadav M, Duerr S, Schubert S, Fischer H, Stappert D, Wantia N, Rodriguez N, Wagner H, *et al.*: **Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins.** *Nat Med* 2008, **14**:399-406.

- * 64. Yadav M, Zhang J, Fischer H, Huang W, Lutay N, Cirl C, Lum J, Miethke T, Svanborg C: **Inhibition of TIR domain signaling by TcpC: MyD88-dependent and independent effects on *Escherichia coli* virulence.** *PLoS Pathog* 2010, **6**:e1001120.
- * This study examined the mechanism by which the UPEC Toll/interleukin-1 receptor (TIR) homologous protein TcpC modifies the innate immune response during experimental UTI. TcpC is shown to promote UTI-associated pathology through the inhibition of TIR domain signaling and downstream pathways.
- ** 65. Wang Z, Humphrey C, Frilot N, Wang G, Nie Z, Moniri NH, Daaka Y: **Dynamin2- and endothelial nitric oxide synthase-regulated invasion of bladder epithelial cells by uropathogenic *Escherichia coli*.** *J Cell Biol* 2011, **192**:101-110.
- ** The authors show that deficiency in the autophagy gene ATG16L1 is associated with a potent proinflammatory cytokine response with increased recruitment of monocytes and neutrophils to UPEC-infected bladders. The results indicate that Atg16L1 deficiency can confer protection against acute and latent UPEC infection, which has implications for understanding the etiology of UTI.
66. Hilbert DW, Paulish-Miller TE, Tan CK, Carey AJ, Ulett GC, Mordechai E, Adelson ME, Gyax SE, Trama JP: **Clinical *Escherichia coli* isolates utilize alpha-hemolysin to inhibit *in vitro* epithelial cytokine production.** *Microbes Infect* 2012, **14**:628-638.
67. Wang C, Mendonsa GR, Symington JW, Zhang Q, Cadwell K, Virgin HW, Mysorekar IU: **Atg16L1 deficiency confers protection from uropathogenic *Escherichia coli* infection *in vivo*.** *Proc Natl Acad Sci U S A* 2012, **109**:11008-11013.

68. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, *et al.*: **Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 β production.** *Nature* 2008, **456**:264-268.
69. Chen TC, Tsai JP, Huang HJ, Teng CC, Chien SJ, Kuo HC, Huang WS, Chen CN: **Regulation of cyclooxygenase-2 expression in human bladder epithelial cells infected with type I fimbriated uropathogenic *E. coli*.** *Cell Microbiol* 2011, **13**:1703-1713.
- * 70. Loughman JA, Hunstad DA: **Induction of indoleamine 2,3-dioxygenase by uropathogenic bacteria attenuates innate responses to epithelial infection.** *J Infect Dis* 2012, **205**:1830-1839.
- * The authors show that expression of indoleamine 2,3-dioxygenase (IDO) in human uroepithelial cells, neutrophils, and in murine bladder, induced by UPEC, attenuates innate responses to epithelial infection. In UPEC-infected IDO-deficient mice, increased local inflammation in the bladder correlated with reduced survival of bacteria. These data identify a novel pathogen strategy to create local immune privilege at epithelial surfaces, attenuating innate responses to promote infection.
71. Cesario A, Rocca B, Rutella S: **The interplay between indoleamine 2,3-dioxygenase 1 (IDO1) and cyclooxygenase (COX)-2 in chronic inflammation and cancer.** *Curr Med Chem* 2011, **18**:2263-2271.
72. Totsika M, Beatson SA, Holden N, Gally DL: **Regulatory interplay between pap operons in uropathogenic *Escherichia coli*.** *Mol Microbiol* 2008, **67**:996-1011.
73. Hawn TR, Scholes D, Li SS, Wang H, Yang Y, Roberts PL, Stapleton AE, Janer M, Aderem A, Stamm WE, *et al.*: **Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women.** *PLoS One* 2009, **4**:e5990.

74. Tabel Y, Berdeli A, Mir S: **Association of TLR2 gene Arg753Gln polymorphism with urinary tract infection in children.** *Int J Immunogenet* 2007, **34**:399-405.
75. Karoly E, Fekete A, Banki NF, Szebeni B, Vannay A, Szabo AJ, Tulassay T, Reusz GS: **Heat shock protein 72 (HSPA1B) gene polymorphism and Toll-like receptor (TLR) 4 mutation are associated with increased risk of urinary tract infection in children.** *Pediatr Res* 2007, **61**:371-374.
76. Yin X, Hou T, Liu Y, Chen J, Yao Z, Ma C, Yang L, Wei L: **Association of Toll-like receptor 4 gene polymorphism and expression with urinary tract infection types in adults.** *PLoS One* 2010, **5**:e14223.
77. Ragnarsdottir B, Jonsson K, Urbano A, Gronberg-Hernandez J, Lutay N, Tammi M, Gustafsson M, Lundstedt AC, Leijonhufvud I, Karpman D, *et al.*: **Toll-like receptor 4 promoter polymorphisms: common TLR4 variants may protect against severe urinary tract infection.** *PLoS One* 2010, **5**:e10734.
78. Andersen-Nissen E, Hawn TR, Smith KD, Nachman A, Lampano AE, Uematsu S, Akira S, Aderem A: **Cutting edge: Tlr5^{-/-} mice are more susceptible to *Escherichia coli* urinary tract infection.** *J Immunol* 2007, **178**:4717-4720.
79. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, Ghosh S: **A toll-like receptor that prevents infection by uropathogenic bacteria.** *Science* 2004, **303**:1522-1526.
80. Hidmark A, von Saint Paul A, Dalpke AH: **Cutting Edge: TLR13 Is a Receptor for Bacterial RNA.** *J Immunol* 2012, **189**:2717-2721.
81. Barber GN: **Cytoplasmic DNA innate immune pathways.** *Immunol Rev* 2011, **243**:99-108.
82. Franchi L, Munoz-Planillo R, Nunez G: **Sensing and reacting to microbes through the inflammasomes.** *Nat Immunol* 2012, **13**:325-332.

Figure 1.



UPEC virulence factors and innate immune responses that help to shape the pathogenesis and severity of UTI. Increased severity of disease such as the transition from cystitis to bacteremia is associated with immunopathology that stems from severe inflammation, and this underlies the pain and certain symptoms of acute infection. Decreased severity of infection, on the other hand, is associated with less severe inflammatory responses. UPEC virulence factors directly influence the extent of innate immune responses and determine potential lifestyles of the pathogen within the host environment.

BOX 1

Adherence to host cells, a critical first step in UPEC infection, is mediated by fimbriae.

- Chaperone-Usher (CU) fimbriae are encoded by a cluster of genes under the control of the same promoter (operon). CU fimbrial operons typically consist of a gene set encoding an outer membrane usher and a periplasmic chaperone, flanked by one or more genes encoding structural subunits.
- Many UPEC strains contain >10 CU fimbrial operons. These are either located on the chromosome backbone or on mobile genetic elements, such as pathogenicity islands and plasmids.
- Expression of some CU fimbriae in UPEC is phase variable, resulting in heterogeneous bacterial populations with respect to adhesin production.
- CU fimbriae are hierarchically displayed at the bacterial cell surface via regulatory cross-talk. This aids the temporal and spatial colonisation of distinct niches during infection. For example, expression of P fimbriae in UPEC turns off type 1 fimbriae (negative cross-talk), while it sequentially turns on other P-related fimbrial operons (positive cross-talk) [72].
- Regulatory cross-talk also takes place between CU fimbriae and other surface organelles of UPEC, such as flagella, capsule and the autotransporter protein antigen 43.

BOX 2

Innate pattern recognition events involving multiple TLR signalling pathways are associated with susceptibility and resistance to bacterial UTI.

- Single-nucleotide polymorphisms (SNPs) in PRR genes are associated with UTI. Examples: a TLR1_G1805T SNP with protection from pyelonephritis [73]; TLR2_R753Q [74] and TLR4_A896G SNPs with increased risk for UTI in children and women [75,76]; and a TLR5_C1174T SNP with recurrent UTI [73].
- TLR4 promoter variants, which are linked to reduced expression of TLR4 and reduced innate immune responses, are associated with asymptomatic bacteriuria [77].
- Mice deficient in PRR genes reveal the functional inputs of TLRs for determining severity of UTI. Examples: TLR4 knockout (KO) mice develop asymptomatic bacteriuria instead of acute UTI [43]; TLR5 KO mice are highly susceptible to UPEC infection [78]; and TLR11 KO mice are more prone to upper UTI [79].
- Nucleic acid-sensing PRRs such as TLR9, TLR13, STING and AIM2 may have a role in UTI; these are activated by bacterial DNA/RNA [80,81].
- Nucleotide-binding oligomerization domain-like receptors (NLRs) including NLRP1, NLRP3 and NLRC4 respond to pathogen products such as toxins (e.g. α -hemolysin) and flagellin [82], but their roles in UTI have not been analyzed.