Toll-like Receptors and Renal Bacterial Infections

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Urinary tract infection and pyelonephritis are mainly due to uropathogenic *Escherichia coli* (UPEC), and are common infectious diseases that constitute a significant cause of morbidity and mortality in humans. They are also the most frequent infectious complications in renal transplant patients, and can impair long-term renal graft function and outcome. UPEC may invade the kidneys via the systemic circulation or by local retrograde infection. They induce the proinflammatory mediators, which are intended to defend the host and clear bacteria from the kidneys. The Toll-like receptors (TLRs) play a key role in the recognition of bacterial components and in inducing the inflammatory response that is mediated by various intracellular signaling pathways. To date, 13 TLRs have been identified in mammals. Recent studies have provided evidence suggesting that renal tubule epithelial cells express most of the TLRs initially identified in bone marrow-derived cells. Murine renal tubule cells express TLR1, 2, 3, 4, 6, and 11. TLR4, which recognizes lipopolysaccharide (LPS), the main constituent of Gram-negative bacteria, plays a key role in inducing the inflammatory responses elicited by UPEC. This review will consider some aspects of TLR function in the kidney, particularly in the renal tubule epithelial cells, and the role of these receptors in enabling the body to cope with urinary tract infections and pyelonephritis caused by UPECs. *(Chang Gung Med J 2008;31:525-37)*

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Renal bacterial infections are common infectious diseases that can impair renal function and/or lead to renal tubulointerstitial nephritis. Bacteria can invade the kidneys via the systemic circulation or by local retrograde infection. They can cause severe renal dysfunction, and are associated with various kidney diseases, such as IgA nephropathy, renal vasculitis, and lupus nephropathy in post-infectious glomerulonephritis.[1,2] The innate immune system recognizes conserved microbial or viral components, known as pathogen-associated molecular patterns (PAMPs), *via* a limited number of pattern-recognition receptors (PPRs), which constitute the front line of defense against pathogens. In recent years, Toll-like receptors (TLRs) have been shown to play a key role in recognizing PAMPs and initiating innate immunity. A new flush of knowledge about the function of the TLRs has been derived from studies of different lines of TLR-deficient mice generated by gene targeting.[3]

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The involvement of TLRs in immunostimulatory responses was first reported in *Drosophila*. The Toll protein receptor involved in establishing dorsoventral polarity has been shown to play a critical role in defending *Drosophila* against fungal infections.[4] Since this major discovery, 13 TLRs have been identified in mammals. TLRs 1-9 are expressed in both mice and humans. TLR10 is expressed only in humans, whereas TLR11 is only expressed in mice. TLRs are type-1, integral, membrane glycoproteins with a leucine-rich repeat (LRR) domain in their extracellular domain, and a Toll/interleukin-1 (IL-1) receptor (TIR) domain in their intracellular domain.[3,5] The extracellular domain interacts directly or indirectly with microbial ligands, and the TIR domain recruits adaptor signaling molecules.[3-6] TLRs form dimers. For example, the TLR1 and TLR2 heterodimer senses bacterial triacylated lipopeptides. TLR2 can also heterodimerize with TLR6, and this dimer also recognizes bacterial diacylated lipopeptides.

Recognition of bacterial or non-bacterial PAMPS ligands by specific TLRs leads to the activation of transcription factors, such as NF-κB, and members of the interferon (IFN)-regulatory factor (IRF) family.[6] All TLRs contain a TIR domain located in their cytosolic domain. Ligand binding induces homodimerization or heterodimerization of TLRs, and the recruitment of adaptor molecules.[6] All known TLRs, apart from TLR3, interact with adaptor myeloid differentiation factor 88 (MyD88). TLR2 and TLR4 exhibit the MAL coadaptor (also known as TIRAP) required to activate NF-κB. The recruitment of MyD88 facilitates the association of TIR with IL-1-receptor-associated kinases (IRAKs). The phosphorylated IRAKs then dissociate, and interact with TNF receptor-associated factor 6 (TRAF6) to activate transforming growth factor β-activated kinase 1 (TAK1). TAK1 then stimulates the mitogen-activated protein kinase (MAPK) pathways, and induces downstream phosphorylation of the IκB inhibitor proteins by IκB kinases (IKKs), their dissociation and subsequent degradation, which then allows NF-κB to translocate into the nucleus.[7,8] This pathway is the classical MyD88-dependent pathway. However, the NF-κB pathway can also be activated in the absence of MyD88. Under certain circumstances, TLR3 and TLR4 may activate a MyD88-independent NF-κB signaling pathway that leads to the induction of IFN-inducible genes and promotes the maturation of dendritic cells. The adaptor TIR domain-containing, adaptor-inducing INF-β (TRIF) plays a key role in activating this MyD88-independent NF-κB pathway. TRIF interacts with the TIR domain of TLR3, and indirectly interacts with TLR4 *via* the coadaptor TRAM (also known as TICAM).[6]

The glycosylphosphatidylinositol-anchored CD14 protein is also required for the TLR2-TLR6 activated MyD88-dependent pathway, and the LPS-activated TRIF-mediated signaling pathway.[9,10] Fig. 1 shows schematic representation of the various adaptor proteins and TIR-dependent signaling processes that lead to the activation of the transcription factor NF-κB and/or IRFs.

This review will summarize the main functions of TLRs, their expression in the kidneys, particularly in renal tubule epithelial cells, and recent findings concerning the role of some of these TLRs in inducing the innate immune response elicited during renal bacterial infection by uropathogenic *Escherichia coli* (UPEC). We shall not discuss here the role of TLR activation in the pathogenesis of systemic immune disorders, interstitial fibrosis, and acute renal failure, which have been discussed in detail in recent reviews.[1,2,11,12] However, we will describe some of the recent findings concerning the mechanisms of TLR activation in renal tubule epithelial cells during ascending urinary tract infections (UTIs), most of which are caused by uropathogenic strains of *Escherichia coli* (*E. coli*).

Intrarenal expression and function of TLRs

TLR2, TLR1 and TLR6

TLR2 recognizes a wide range of microbial products, such as lipoproteins from Gram-negative bacteria, mycoplasma and spirochetes, peptidoglycan and lipoteichoic acid from Gram-positive bacteria, glycoinositolphospholipids from *Trypanozoma Cruzi*, zymosan from fungi, or porins.[3] TLR2 also recognizes atypical LPS from *Leptospira interrogans* and *Porphyromonas gingivalis*, but not those from *E. coli* or *Salmonella spp*., which are ligands for TLR4.[13,14] In the kidney, TLR2 expressed in the renal tubule proximal cells recognizes Leptospiral outer membrane proteins, and this leads to the activation of NF-κB, and mitogen-activated protein kinases (MAPKs).[15] However, differential recognition of purified Leptospiral lipid A by TLRs is known to

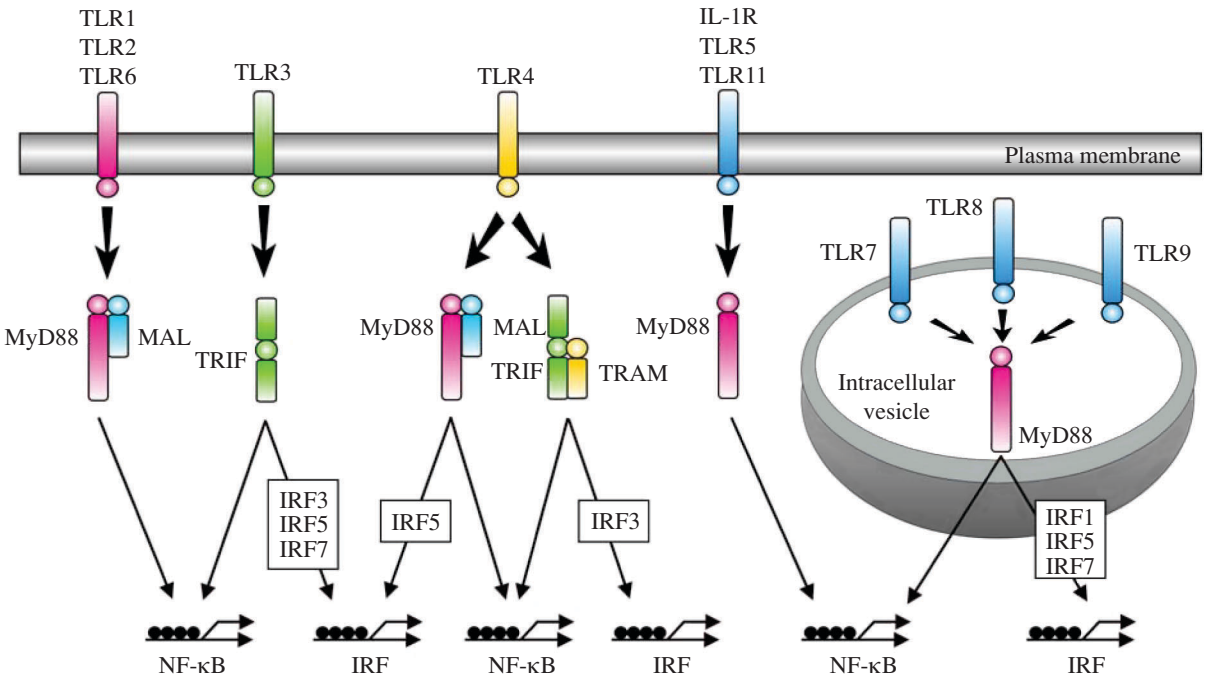

Fig. 1 Schematic representation of adaptor molecules associated with the TIR domain of Toll-like receptors (TLRs) (from ref. 6). IL-1R: interleukin-1 receptor; IRF: interferon regulatory factor; MyD88: myeloid differentiation factor 88; MAL: MyD88-adaptor-like; TRIF: MyD88 and Toll IL-1 receptor (TIR) domain-containing adaptor-inducing IFN-beta (TRIF); TRAM: Trif-related adaptor molecule.

exist. TLR2/TLR1 have been shown to be the predominant receptor in human cells, whereas TLR2 and TLR4 both contribute to cell activation in murine macrophages.[13,16] TLR2 also recognizes various endogenous ligands including Hsp70, which is upregulated following ischemia/reperfusion (I/R) injury,[17] and which may play a role in activating TLR2 in ischemic tissues.

TLR2 interacts with the highly homologous receptors TLR1 and TLR6 to discriminate between different microbial components. For example, TLR1 and TLR2 both signal soluble factors released from *Neisseria meningitidis*.[18] TLR1 also plays an important role in the recognition of triacyl lipopeptides.[19] Interestingly TLR2, which is upregulated during renal I/R injury,[17] plays a key role in inducing the inflammatory response and cell damage.[20] TLR2-deficient mice exhibit a much lower inflammatory response, less leukocyte infiltration and consequently less renal tubule cell damage than their wild-type mice counterparts when subjected to I/R. Moreover, *in vivo* injection of TLR2-antisense RNA has also been shown to produce effective protection against the kidney dysfunction caused by I/R.[20] Shigeoka *et al*.[21] have also demonstrated that the induction of the inflammatory response mediated by TLR2 occurs via both MyD88-dependent and MyD88-independent, TRIF-dependent pathways. However, the exact mechanism by which TLR2 is activated during I/R injury still remains largely unresolved.

TLR3

TLR3 recognizes the single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA) produced by many viruses during replication.[3] Expression of human TLR3 in double-stranded RNA-unresponsive cells allows NF-κB to be activated by double-stranded RNA.[22] TLR3 is distinguished from the other TLRs in not having a proline residue that is conserved in the other TLRs. This residue corresponds to the proline residue that is mutated in the *tlr4* gene from LPS-defective (also designated *Lps*^{d} mice) C3H/HeJ mice,[23] which are unresponsive to LPS and fail to clear Gram-negative bacteria colonizing the lower urinary tract and kidneys.[24] Human TLR3 has been shown to be prefer-

entially expressed in mature dendritic cells.[22,25] TLR3 mRNA has also been shown to be expressed in human kidneys.[25,26] Moreover, TLR3 has also been found to be expressed in renal mesangial cells, together with infiltrating APCs, in an experimental murine model of lupus.[27] It has been suggested that TLR3 may be implicated since dsRNA is known to activate dendritic cell cytokines and type-I interferons that are associated with systemic lupus erythematosus (SLE). Viral dsRNA has been shown to exacerbate lupus-induced nephritis in MRLlpr/lpr mice, which spontaneously develop immune complex glomerulonephritis.[27]

TLR4

TLR4 is the main receptor for LPS from Gram-negative bacteria. Since the early 1980s, it has been known that some strains of mice, such as the C3H/HeJ strain, are highly sensitive to UTIs and fail to clear bacteria. Ten years later, two studies identified a point mutation in the *tlr4* gene in the LPS-defective C3H/HeJ mouse strain, and a null-mutation on the *tlr4* gene in LPS-hyporesponsive C57BL10/ScCr mice.[23,28] TLR4-deficient mice, produced by targeted disruption of the *tlr4* gene, exhibit the same LPS-hyporesponsive phenotype, confirming that TLR4 is the receptor for LPS.[29] TLR4 mutations linked with hyporesponsiveness to LPS have been also identified in humans.[29]

The recognition of LPS by TLR4 requires the presence of two other molecules, CD14 and MD-2. CD14 is thought to interact with TLR4 in LPS signaling[30,31] whereas MD-2 associates with the extracellular domain of TLR4, and enhances LPS-induced cell activation.[32,33] The RIP105 protein, which is preferentially expressed on the cell surface of B cells, is also involved in recognizing LPS.[34] Taken together, these studies indicate that TLR4 forms a large complex with several associated proteins to acheive efficient LPS-induced cell activation.

TLR4 also recognizes the other ligands listed in Fig. 2. One of them, taxol, a product of the Pacific yew (*Taxus brevifolia*) which exhibits potent antitumor activity, also induces a potent TLR4-MD-2-mediated inflammatory response.[35] The heat shock proteins Hsp60 and Hsp70 have also been shown to activate TLR4-mediated NF-κB and MAP kinase pathways.[36] TLR4 also recognizes Hsp70, which is over-expressed in ischemic renal tubule cells (17). However, the role of Hsp in TLR activation remains controversial due to the possibility of contamination of purified Hsp with LPS.[37] A recent study which demonstrated that TLR4, like TLR2, plays an active role in initiating an inflammatory response and renal tubule cell apoptosis, also showed that Hsp70 is not activated in the kidneys of mice subjected to I/R injury.[38] Components of the extracellular matrix, such as fibronectin, hyaluronic acid or heparan sulfate, are released during cell injury and also activate TLR4 and TLR2. The extracellular domain A of fibronectin, soluble heparan sulfate, oligosaccharides of hyaluronic acid, and β-defensin 2 have also been shown to activate TLR4.[1,3] It should be noted that these endogenous TLR4 ligands only activate immune cells at high concentrations, which contrasts with the cell activation produced by low concentrations of LPS. Furthermore, it is impossible to exclude the possibility that these ligands may have been contaminated by LPS.

TLR5

TLR5 recognizes monomeric flagellin, the primary protein component of the flagellum, a highly complex structure protruding from the outer membrane of Gram-negative bacteria.[39] Bacteria use their flagella to propell themselves in aqueous environments. The flagella also play a role in attaching the bacteria to host cells, and have been shown to contribute to the virulence of pathogenic bacteria. Flagellin induces a potent immune response in both mammalian and plant cells.[40] However, some bacteria, such as *Helicobacter pylori* and *Bartonella bacilliformis,* exhibit modified flagellin which does not elicit proinflammatory responses.[41] TLR5 is highly expressed in intestinal epithelial cells. TLR5 engagement by bacterial flagellin induces cell activation, leading to the production of IL-8 and the macrophage inflammatory protein 3α[42] Flagellin has also been shown to be the main determinant of *Salmonella*-mediated NF-κB activation of proinflammatory signaling.[43] TLR5 located in the basolateral membranes of intestinal epithelial cells is also able to discriminate between commensal and pathogenic flagellated bacterial strains; basolaterally-located, pathogenic, flagellated bacteria induce an inflammatory response via TLR5-mediated signaling.[44,45] *In vivo* exposure of dextran sulfate-injured colon, but not of intact colon, to flagellin significantly exacerbates

TLR	Ligands	TLR expression in renal tubule cells
TLR1	Triacyl lipopeptides	Cultured RTECs*
TLR2	Lipoprotein/lipopeptides Peptidoglycan Lipoteichoic acid, glycolipids Porins, Zymosan A typical LPS (*Leptospira interrogans*) Heat shock proteins	Bowman's capsule parietal cells PCT, TD, CD
TLR3	Double-stranded RNA (virus)	Mesangial cells CD
TLR4	LPS (Gram-negative bacteria) Taxol, fusion protein, envelope proteins Heat shock proteins, fibronectin Hyaluronic acid, heparan sulfate Fibrinogen, β-defensin 2	Bowman's capsule parietal cells PCT, TAL, DT, CD
TLR5	Flagellin	?
TLR6	Diacyl lipopeptides, lipoteichoic acid Zymosan	Cultured RTECs*
TLR7	Imidazoquinoline, loxoribine Single-stranded RNA	Not expressed
TLR8	Imidazoquinoline, loxoribine Single-stranded RNA (virus, bacteria)	Not expressed
TLR9	CPG-DNA (bacteria) Single-stranded RNA (virus, bacteria)	Not expressed
TLR10	?	
TLR11	Profilin-like protein (*Toxoplasma gondii*)	RTECs*

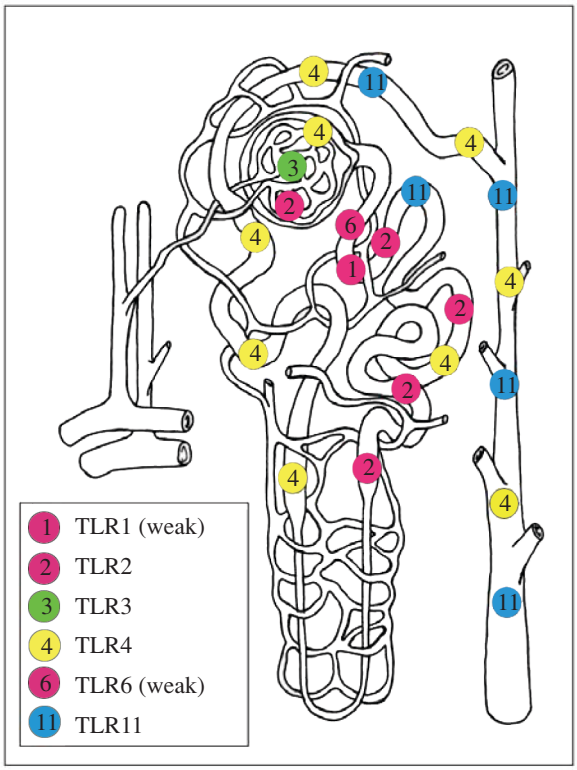

Fig. 2 Toll-like receptor (TLR) ligands and renal tubule cell expression of TLRs. Summary of the TLR ligands recognized by TLRs and schematic representation of the intrarenal distribution of TLRs along the nephron (from refs 1, 5 and 11). PT: proximal tubule; TAL: thick ascending limb; DT: distal tubule; CD: collecting duct; RTECs: renal tubule epithelial cells; *: Specific tubule cell expression not determined.

colonic inflammation, indicating that flagellin plays an important role in the development and progress of colitis.[46] However, the polarity of the expression of TLR5 remains controversial, since apical expression of TLR5 has also been reported in cultured intestinal-like HT29 cells and in murine intestine.[47] A stop codon polymorphism in the ligand-binding domain of TLR5 acting in a negative dominant fashion is associated with increased susceptibility to flagellated *Legionella pneumophila*, which causes pneumonia in humans. It has been suggested that TLR5 may play an essential role in mediating an innate immune response in lung epithelial cells.[48] TLR5 senses *Pseudomonas aeruginosa* infections in the epithelial cells of the airways.[49] A recent study has also demonstrated that TLR5 mediates an innate immune inflammatory response in bladder and kidneys infected by *E. coli*,[50] which indirectly suggests that TLR5 is expressed in kidney epithelial cells. However, TLR5 mRNA expression has been detected in primary cultures of mouse renal cortical tubule cells,[51] and we cannot rule out the possibility that the expression of TLR5 remains restricted to some specialized renal tubule cells.

TLR7, TLR8, and TLR9

TLR7 and TLR8 are highly homologous with TLR9. They are expressed in the endoplasmic reticulum and in intracellular endosomal organelles. TLR7, TLR8, and TLR9 all recognize nucleic acids. TLR7 and TLR8 can sense viral single-stranded RNA (ssRNA) from viruses and bacteria,[52] and synthetic imidazoquinolines, which are known to exhibit potent antiviral and antitumor properties. The synthetic nucleoside analog, R488, is also a ligand for TLR7 and TLR8.[53] TLR7 and TLR8 have not been

detected in kidney epithelial cells. TLR9 recognizes unmethylated 2'-deoxyribo (cytidine-phosphate-guanine) (CpG) motifs that are found in DNA from viruses and bacteria, but not in DNA from eukaryotes.[54] CpG DNA stimulates the proliferation of B cells, and the secretion of proinflammatory cytokines required to eliminate invading pathogens.[55,56] For example, CpG DNA protects mice against infections caused by intracellular pathogens such as *Leishmania major* and *Listeria monocytogennes*.[57-59] TLR9 is expressed in B cells, dendritic cells and monocyte/macrophages, and is localized in the endoplasmic reticulum of resting cells. It becomes activated after being translocated from the endoplasmic reticulum to endocytotic CpG DNA in the lysosomes.[60] TLR9 is also expressed in epithelial cells including gastric cells and intestinal epithelial cells.[61,62] In intestinal cells, DNA from pathogenic strains of *Salmonella* and *E. coli* stimulates TLR9 mRNA expression.[63] Lee *et al*.[64] demonstrated that TLR9 activated polarized intestinal epithelial cells differently when added to the apical or basolateral side of cells grown on filters. These authors showed that the apical expression of TLR9 signals the degradation of IkBα and the concomitant activation of NF-κB, whereas the apical expression of TLR9 limits inflammatory responses following subsequent stimulation of TLR9 by a mechanism in which ubiquinated IkBα accumulates in the cytoplasm, thereby preventing NF-κB activation.[64] With regard to the kidney, TLR9 mRNA has not been detected in cultured renal tubule cells.[53] However, this does not completely exclude the possibility that TLR9 may be restricted to some specialized tubule epithelial cells. TLR9 is thought to be involved in the pathogenesis of SLE by activating B cells and stimulating the production of cytokines. The implication of TLR7, and the role of TLR9 in the progression of SLE has been discussed in recent reviews.[2,11]

TLR11

TLR11 is expressed in mice but not humans. TLR11 recognizes the profilin-like protein from *Toxoplasma gondii*.[65] Profilin belongs to the group of small actin-binding proteins that play a role in actin polymerization, suggesting that TLR11 in mice might be involved in the transfer and carriage of the parasite. TLR11 initiates signal transduction that leads to the activation of NF-kB and AP-1 in HEK 293 cells expressing CD14-TLR11.[66] However, its exact function still remains to be determined. Interestingly, TLR11 has also been shown to be involved in sensing uropathogenic *E. coli* in mice.[66] TLR11 is predominantly expressed in the epithelial cells of the bladder and kidneys. However, no colocalization studies with renal tubule cell specific markers have been performed.[66]

Cellular and subcellular localization of TLRs in renal tubule epithelial cells

Mouse tubule epithelial cells express TLR1, TLR2, TLR3, TLR4 and TLR6 mRNA.[53] mRNA expression by complete TLRs has been reported in human kidneys, but without discriminating between TLR expression from tubule epithelial cells and that from circulating immune cells.[67,68] Fig. 2 summarizes the main exogenous and endogenous ligands recognized by TLRs, and the intrarenal distribution of the TRLs expressed in renal tubule cells.

TLR1. Although renal tubule epithelial cells have been shown to express TLR1, its exact location within the tubule cells is not known.

TLR3. TLR3 is expressed in myeloid dendritic cells,[23,25,26] and has also been found to be expressed in murine and human kidneys.[22,67] TLR3 expression in renal mesangial cells and immune cells that have infiltrated the kidneys has also been demonstrated in an experimental murine model of lupus.[27] Analysis of TLR3 mRNA expression in human renal biopsies has also revealed that it is expressed in the renal mesangium and collecting duct cells.[69]

TLR2 and ***TLR4***. These two TLRs are highly expressed in bone marrow-derived cells, and also in a variety of non-epithelial cells, including endothelial cells, smooth-muscle cells and intestinal epithelial cells. Predominantly TLR2 mRNA expression has been demonstrated by *in situ* hybridization in the proximal tubule and distal-collecting tubule.[70] Immunofluorescence studies performed in mouse and rat kidneys detected the TLR2 protein in the proximal tubule, thick ascending limb cells and distal tubules.[17,70] TLR2 expression is also present in glomerular cells, possibly in the mesangial cells, and along Bowman's capsule.[21] Interestingly, TLR2 is localized in the basolateral membranes of intact renal tubule cells, but remains mainly in the cytoplasm of ischemic renal tubule cells.[21]

TLR4. The exact subcellular distribution of

TLR4 in epithelial cells is still controversial. TLR4 has been shown to be expressed at both the mRNA and protein levels in mouse, rat and human renal tubule cells. *In situ* hybridization revealed the presence of TLR4 mRNA in the proximal tubule, thick ascending limb and distal-collecting tubule and in Bowman's capsule.[17,71,72] TLR4 is highly expressed at the cell surface of macrophages, and it has also been reported to be intracellularly located in a variety of epithelial and non-epithelial cells. Cultured murine intestinal crypt cells express TLR4 exclusively in the Golgi apparatus.[73] The localization of TLR4 in renal tubules still remains a matter of debate. TLR4 has been identified in the brush-border membranes of rat proximal tubule cells.[72] Using an antiserum raised against mouse TLR4, Chassin *et al*.[74] showed that TLR4 is mainly localized in the cytoplasm of intact renal tubule cells, and mainly expressed in the thick ascending limb cells and collecting duct cells. Furthermore, immunohistochemical analysis of wild-type kidneys from mice 2 days post-infection with UPECs revealed that TLR4 colocalized with the internalized UPECs mainly in the cytoplasm of collecting duct intercalated cells.[74] A recent study from our laboratory also demonstrated that TLR4 was located in the Golgi apparatus, and colocalized with the Golgi apparatus marker CTR433 and with p58K (in preparation). Further studies are therefore required to clarify the subcellular distribution of these TLRs in non-stimulated and stimulated renal epithelial cells.

TLR5. TLR5 mRNA expression has not been detected in murine tubule epithelial cells. As TLR5-deficient mice have been shown to be more vulnerable to retrograde UPEC infection,[50] TLR5 expression in renal tubule cells cannot be excluded.

TLR11. *In-situ* hybridization demonstrated that TLR11 is expressed in renal tubule cells.[72] However, no immunohistochemical studies have been undertaken to analyze the intrarenal distribution of the TLR11 protein.

TLRs and urinary tract infection

Urinary tract infections (UTIs), including asymptomatic bacteriuria, cystitis and pyelonephritis, are among the most common infectious diseases and constitute a major cause of human morbidity and mortality.[75,76] Furthermore, acute or chronic pyelonephritis can lead to severe renal damage, which triggers the onset of end-stage renal failure.[77] UTIs are also the most common form of bacterial infection in renal transplant recipients.[78,79] It is generally agreed that post-transplant UTIs are caused by exposure to pathogens as a result of surgical procedures (i.e. urethral and ureteral stent catheters) and long-term immunosuppressive therapy.[79,80] Until recently, UTIs have been considered to be relatively benign. However, we have recently shown that acute pyelonephritis (APN) could be an independent risk factor associated with an enduring decline of renal graft function,[81] suggesting that intrarenal infection, which tends to promote renal scarring, may be deleterious for the maintenance of long-term renal graft function. UPECs are the main microorganisms responsible for UTIs.

Type-1 and type-P fimbrial adhesins expressed on the surface of UPEC play central roles in bacterial attachment to mucosal epithelial cells,[82] which is the first step in *E. coli* pathogenicity. The binding of P-fimbriated and type-1 adhesins to epithelial cell receptors determines tissue specificity, and allows UPEC to ascend into the lower urinary tract and the kidneys. The recognition of UPEC by the mucosal cells lining the urinary tract triggers a potent inflammatory response in a process involving TLR4. Studies using experimental models of ascending UTIs in mice and human bladder and renal tubule epithelial cells have provided clear evidence that the renal inflammatory response to type-P and type-1 fimbriated *E. coli* is indeed TLR4 -dependent.[83-85] A recent study has also shown that type-P and type-1 fimbriated *E. coli* may utilize different adaptor molecules to influence neutrophil activation and bacterial clearance, but that in both cases MyD88 is required for efficient bacterial clearance.[86]

Recent studies have provided evidence that the renal tubule epithelial cells play a critical role in inducing the innate response during UTI. Using hematopoietic chimeric Lps^n and Lps^d mice to compare the specific contributions of TLR4 expressed by hematopoietic cells and parenchymatous bladder epithelial cells, Schilling *et al*.[87] have demonstrated that bladder epithelial cells expressing TLR4 can actively clear bacteria during an acute infection, but that the presence of TLR4-expressing hematopoietic cells is required to elicit a full innate immune response. Using a similar approach, Patole *et al*.[88] have also reported that TLR4 is required in both

intrinsic renal tubule cells and bone marrow-derived cells to initiate chemokine-driven, renal neutrophil recruitment. Chassin *et al*.[74] demonstrated that UPECs invading kidneys specifically bind to the apical surface of collecting duct cells, mainly to intercalated cells (Fig. 3 A and B). The signaling pathways activated by UPEC in collecting duct cells have also been investigated in primary cultures of medullar collecting duct cells dissected from the kidneys of LPS-sensitive C3H/HeOuJ mice expressing a functional TLR4, of LPS-defective C3H/HeJ mice, and of mice deficient in MyD88 or TRIF.[74] Analysis of signaling pathways revealed that UPECs stimulated the expression of proinflammatory mediators in the medullary collecting ducts via TLR4-mediated, MyD88-dependent, TRIF-independent NF-kB and MAPK activated pathways, and also via a TLR4-independent, MyD88-independent pathway. This latter TLR4 pathway results from activation of the TNF receptor-associated factor-2 (TRAF2), apoptosis signal-regulatory kinase 1 (ASK1)-JNK pathway.[74] Fig. 3C summarises the different signaling pathways activated by UPECs in renal medulllary collecting duct cells.

The fact that the collecting duct system is the main site of adhesion (and perhaps of internalization) of UPEC raised the possibility that the hormones involved in controlling sodium and water reabsorption, and that specifically act on the collecting duct, could be involved in controlling the inflammatory response elicited by collecting duct cells. Activation of cAMP mediated signaling has been shown to suppress the immune response elicited by LPS and TNF-α in a variety of cell types.[89-91] These studies suggest that agents which increase cell cAMP content could possibly alter inflammatory signaling and the subsequent production of cytokines/chemokines, thereby altering the host's defenses. We recently reported

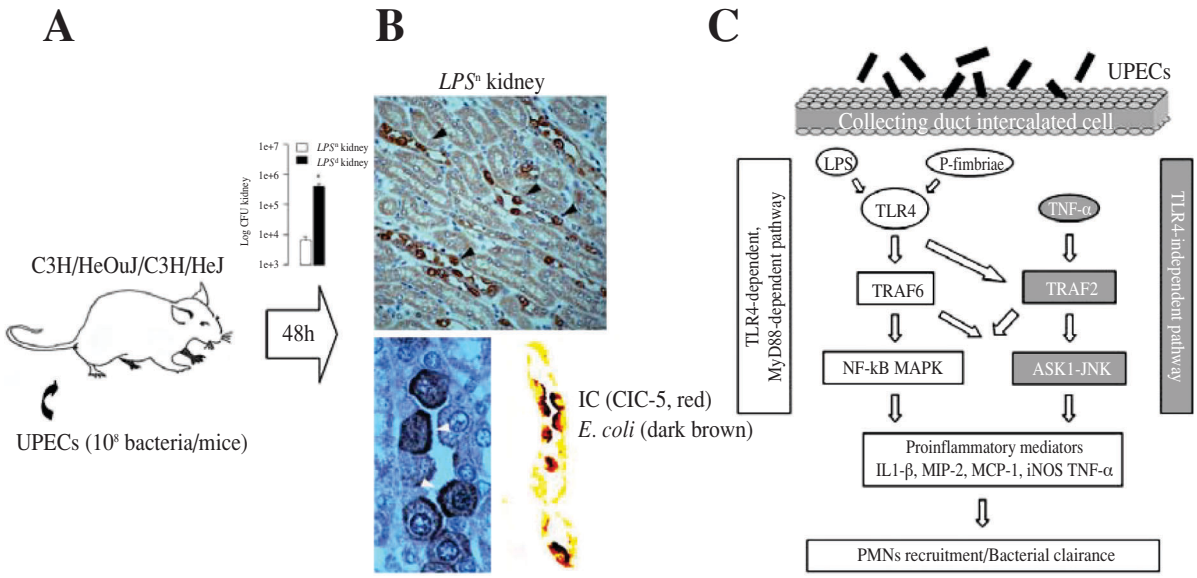

Fig. 3 Medullary collecting tubule cells are the main site of bacterial adhesion and initiation of the inflammatory response elicited by uropathogenic *E. coli* in a mouse experimental model of ascending urinary tract infection. (A) Wild-type C3H/HeOuJ or TLR4-defective C3H/HeJ mice were infected by transurethral inoculation of UPECs. Kidneys were analyzed 48 h after bacterial inoculation.[74] The number of UPECs colonizing kidneys was significantly greater (*, $p < 0.05$) in infected C3H/HeJ mice than in infected C3H/HeOuJ mice. (B) Illustrations of *E. coli* immunostaining (dark brown) restricted to the apical side of some, but not all, sections of tubule cells (arrowheads) identified as intercalated chloride channel ClC5 positive cells (in red).[74] (C) Summary of the TLR-dependent and -independent signaling pathways activated by UPEC in medullary collecting duct cells.[74] ASK1: apoptosis signal-regulating kinase 1; IC: intercalated cell; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; MCP-1: monocyte chemoattractant protein 1; MIP-2: macrophage inflammatory protein-2; PMNs: polymorphonuclear neutropils; TLR4: Toll-like receptor 4; TRAF2: TNF receptor-associated factor 2; TRAF6: TNF receptor-associated factor 6; UPEC: uropathogenic *Escherichia coli*.

that deamino arginine vasopressin (dDAVP), a pure V2 receptor (V2R) analog, which induces an increase in cell cAMP and stimulates water and NaCl absorption by collecting duct cells, acts as a potent inhibitor of the inflammatory response elicited *in vitro* by LPS and *in vivo* by UPEC.[92] This study identified local hormonal control of the renal immune response and bacterial host defense by dDAVP.[92]

It has been suggested that TLR11, in addition to TLR4, may be a receptor for uropathogenic *E. coli* invading the kidneys.[66] The fact that TLR11 is not expressed in humans could explain why humans are highly susceptible to urinary infections. Further studies are now be required to determine the exact role of TLR11 in *E. coli* adhesion to and/or invasion in murine tubule epithelial cells. TLR5 also seems to play a role in renal bacterial infection since Andersen-Nissen *et al*.,[50] using an experimental mouse model of ascending UTI, showed that TLR5-deficient mice had significantly more bacteria in their bladder and kidneys, and paradoxically exhibited a greater inflammatory response, than their wild-type counterparts.

Conclusion

Some of the TLRs that have been identified are expressed in kidneys, both in bone marrow-derived cells and in parenchymal renal glomerular and tubule epithelial cells. Together with circulating immune cells, tubule epithelial cells play a key role in recognizing PAMPs and activating signaling pathways that lead to the production of cytokines/chemokines to attract polymorphonuclear cells to the site of inflammation to ensure efficient bacterial clearance. The release of excessive levels of cytokines, such as TNF-α, may however cause tissue damage, as is the case during LPS-induced acute renal failure which is in part due to important TLR4-mediated production of systemic TNF-α.[93] Conversely, blocking TNF-α has been shown to improve renal function in experimental models of endotoxemic renal failure.[94] In contrast, blocking TNF-α may aggravate the renal bacterial burden and favor the formation of multiple abscesses in kidneys of TLR4-deficient C3H/HeJ mice infected with UPECs.[92] These apparent divergent findings indicate that the stimulation of proinfammatory mediators plays essential roles in the first line of defense against pathogens, but when excessive, may also induce kidney damage. A better understanding of the mechanism controlling the innate immune response during UTI is also of particular interest in some clinical situations. Renal transplant patients are highly vulnerable to UTIs and pyelonephritis. Experimental models of acute I/R injury have been used as models of acute rejection, and provide evidence of the decisive role of TLR2, TLR4 and MyD88 in activating an immune response.[3,20,21,38,70] It has also been suggested that MyD88 may be involved in minor antigen-mismatched skin allograft rejection.[95] Interestingly, two single-nucleotide TLR polymorphisms associated with endotoxin responsiveness have been reported.[96] TLR4 polymorphisms observed in renal transplant recipients have been shown to present a lower risk of atherosclerosis and acute rejection, but these patients experience more frequent severe bacterial and cytomegalovirus (CMV) infections.[97] Another recent study also reported that TLR4 polymorphism was associated with a higher risk of CMV infection, but in contrast with the former study, did not find that this was associated with a greater frequency of bacterial infections.[98] The mechanism responsible for the high frequency of UTIs and pyelonephritis in renal transplanted patients is still not clear. Further studies are required to elucidate the mechanism by which immunosuppressive treatment promotes UTIs and pyelonephritis in renal transplant patients.

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