Contribution of Renal Tubule Epithelial Cells in the Innate Immune Response during Renal Bacterial Infections and Ischemia-reperfusion Injury

Sanae Ben Mkaddem, PhD; Cecilia Chassin, PhD; Alain Vandewalle, MD, PhD

The epithelial cells that line the renal tubule are sometimes severely injured in the course of inflammatory kidney diseases. These renal tubule epithelial cells (RTECs) express some of the Toll-like receptors (TLRs) of the innate immune system. A number of studies have implicated RTECs, together with bone marrow-derived cells, in triggering an innate immune response to bacterial infection and/or ischemic stress. RTECs expressing TLR4, which recognizes lipopolysaccharide (LPS), contribute to defending the host against ascending urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPECs). Activation of TLR2 and TLR4 signaling by endogenous damage-associated molecular patterns controls the inflammatory responses of RTECs and cell apoptosis in kidneys subjected to ischemia/reperfusion (I/R) injury. This review will consider some recent advances in understanding of the role of RTECs in inducing the innate immune response in experimental models of ascending UTIs and renal I/R injury. Arginine vasopressin, which regulates renal water absorption, has been shown to act as a potent modulator of the innate response in collecting duct cells, a preferred intrarenal site for UPEC adhesion. The activation of the mitogen-associated protein kinase ERK1/2 in post-hypoxic RTECs has also been shown to be selectively regulated by TLR2 *via* the serine-threonine protein phosphatase 5, which is associated with the endoplasmic reticulum resident heat shock protein, gp96, which acts as a master chaperone of TLRs. These findings provide further support for the concept that RTECs are actively involved in triggering the innate immune response, at least in the context of ascending UTIs and I/R injury. *(Chang Gung Med J 2010;33:225-40)*

Prof. Alain Vandewalle

Key words: Toll-like receptor, renal tubule epithelial cell, inflammation, urinary tract infection, ischemia-reperfusion injury

Mucosal cells lining the intestinal, lung and urinary tract systems form a physical barrier to pathogens. Epithelial intestinal cells, tracheal and pulmonary alveolar cells, as well as epithelial bladder cells and renal tubule epithelial cells, which have distinct physiological functions, are highly sensitive

From the INSERM U773, Centre de Recherche Biomédicale Bichat-Beaujon (CRB3), Paris, France; Université Paris 7 - Denis Diderot, site Bichat, Paris, France.
Received: Oct. 28, 2009; Accepted: Jan. 7, 2010
Correspondence to: Prof. Alain Vandewalle, INSERM U773, Centre de Recherche Biomédicale Bichat-Beaujon (CRB3). 16 rue Henri Huchard, BP416, F-75870, Paris, France. Tel.: 33-1-57-277550; Fax: 33-1-57-277531; E-mail: alain.vandewalle@inserm.fr

to the development of local and systemic inflammatory diseases. In contrast to those of the intestine and lungs, the epithelial cells lining the upper (i.e. kidney) and lower (i.e. bladder) urinary tract, where the urine is excreted, constitute a sterile environment which is not normally exposed to bacteria. However, in particular circumstances, the bladder and kidney can be colonized by uropathogenic bacteria. Urinary tract infection (UTI), usually occurs *via* retrograde ascent of bacteria. UTIs are the most common bacterial infections in children and adults,[1-3] and are also the most common bacterial complication in renal transplant patients.[4-7] In recent years, much attention has been paid to the urinary tract pathogenicity of *Escherichia coli* (*E. coli*), the emergence of highly-virulent, antibiotic-resistant strains of *E. coli*,[8] the ability of uropathogenic *E. coli* (UPECs) to form biofilms,[9] the development of intracellular bacterial communities of UPECs,[10,11] and the long-term consequences of complicated UTI (i.e acute pyelonephritis, APN) for renal graft function.[4,5,12] The renal mucosal inflammation that results from the interaction between uropathogenic bacteria and/or epithelial bladder or renal tubule cells can lead to asymptomatic bacteriuria, urethritis, cystitis, and acute and chronic pyelonephritis. Immune cells from the hematopoietic compartment, including monocytes, macrophages and neutrophils, play key roles in triggering the innate immune response to defend the host and r in maintaining the hyperinflammatory stress that can lead to destruction of the epithelium. For example, the episodes of acute inflammation in inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis and necrotizing enterocolitis, are all associated with marked impairment of the integrity of the intestinal epithelium.[13] This can also happen during pyelonephritic episodes induced by UPECs, leading to severe mucosal inflammation that is sometimes associated with changes in the renal epithelium.

In the past decade, the identification of the receptors of the innate immune systems, their ligands (known as pathogen-associated mitogen patterns, PAMPs), signaling molecules, and downstream transcription factor NF-κB and mitogen-associated protein (MAP) kinase pathways has been a major step forward in understanding the mechanisms underlying mucosal inflammation. The main pattern recognition receptors of the innate immune system consist of the Toll-like receptors (TLRs),[14,15] the Nacht-LRR (NLR) receptors,[16,17] including Nod1 and Nod2, intracellular proteins that sense the presence of invasive bacteria, the RIG-like receptors (RLRs), and mannose-binding lectins.[18] It has been suggested that immune hematopoietic cells play a predominant role in inducing and controlling the innate immune response during mucosal inflammation. In addition, some recent (albeit somewhat limited) studies have shown that epithelial cells, including intestinal, pulmonary and renal tubule cells, exhibit some (but not all) of the TLRs that have been identified in leukocytes. These studies have also shown that epithelial cells stimulated by TLR agonists or pathogens display potent TLR-mediated inflammatory responses. These findings have led to the emerging concept that epithelial cells may play a critical role in the pathogenesis of mucosal inflammation.[19]

Epithelial cells also sense the presence of endogenous molecules, known as damage-associated molecular patterns (DAMPs), that are released by stressed cells.[20] Recent studies have indicated that stressed hypoxic epithelial cells play a central role in the development of mucosal immunity in tissues subjected to ischemia-reperfusion (I/R) injury. This short review will summarize recent findings about the critical role of TLR signaling by renal tubule cells in the induction and control of the innate immune response during ascending UTIs and cell apoptosis occurring during renal I/R injury.

A role for renal tubule cells in inflammatory kidney diseases

TLR signaling plays a central role in the development of mucosal inflammation. TLRs are type-1 integral membrane glycoproteins with a leucine-rich repeat (LRR) domain in their extracellular domain, and a Toll/IL-1R (TIR domain) homologous with the interleukin-1 (IL-1) receptor.[14] Thirteen TLRs have been identified in both humans and mice. TLR4 was the first TLR to be identified; this receptor recognizes lipopolysaccharides (LPS or endotoxin) from Gram-negative bacteria.[21] TLR/ligand interaction leads to the recruitment and activation of adaptor molecules.[15] We will make no attempt to describe the detailed molecular mechanisms underlying TLR signaling here.[21,22] Briefly, all TLRs, except TLR3, interact with adaptor myeloid differentiation factor 88 (MyD88). TLR2 and TLR4 exhibit the coadaptor

MAL (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 become dissociated and interact with TNF receptor-associated factor 6 (TRAF6) to activate TAK1. Once activated, TAK1 forms a complex with TK1 protein binding proteins, which then leads to the downstream phosphorylation of the IκBs inhibitor proteins by IκB kinases (IKKs), their dissociation and subsequent degradation. This then allows the transcription factor, NF-κB, to translocate into the nucleus.[22,23] Activation of TAK1 also stimulates mitogen-activated protein kinase (MAPK) pathways including extracellular signal-regulated kinase (ERK), p38 MAP kinase, and c-jun N-terminal kinase (JNK).[24-26] Activation of TLR3, and also that of TLR4, also activates the Toll/IL-1 receptor (TIR) domain containing adaptor inducing IFN-β (TRIF)-dependent/MyD88-independent signaling, resulting in the induction of type-1 interferon.[27,28]

All TLRs are expressed in leukocytes, including monocytes, macrophages, dendritic cells, T-and B-cells and neutrophils (except TLR3). TLRs are also expressed in a variety of other non-epithelial and epithelial cell types, including renal tubule cells.[29] Tsuboi et al. first reported the expression of TLRs 1, 2, 4, and 6 in primary cultures of mouse cortical renal epithelial cells.[30] Other studies have confirmed that TLR2 and TLR4 are expressed in renal tubule cells from mouse,[31-35] rat,[36] and human kidneys.[37,38] TLR5 has also been shown to be expressed in the mouse bladder and, to a lesser extent, in mouse kidneys.[39] However, conflicting findings have been reported for the expression of TLR5 by the renal tubule cell. TLR5 is reportedly expressed in cultured human renal epithelial cells,[37] but not in primary cultures of mouse cortical renal tubule cells.[30] TLR11, which is not expressed in humans, is also expressed in murine bladder epithelial cells and renal tubule cells, and is involved in sensing the presence of uropathogenic *E. coli* in mice.[40] TLR11 has also been shown to recognize the profilin-like protein from *Toxoplasma gondii*.[41] Table 1 summarizes the TLRs expressed in human and murine renal tubule epithelial cells, and their corresponding PAMP ligands. Fig. 1 is a diagram showing the main adaptor signaling molecules associated with the TLRs expressed in renal tubule epithelial cells.

Table 1. PAMP Ligands Related to TLRs Expressed in Renal Tubule Epithelial Cells

TLR	Tubule cell expression	Exogenous PAMP ligands
1	ARN	Triacyl lipopeptides
2	ARN, protein	Lipoproteins/lipopeptides Lipoteichoic acid Glycolipids Porins Atypical LPS (*Leptospira*) Zymosan
3	ARN	Double stranded RNA (virus) LPS Taxol
4	ARN, protein	Retroviral envelop proteins Microbial Hsp
5	ARN	Flagellin
6	ARN	Diacyl lipopeptides Lipoteichoic acid Zymosan Double-stranded RNA
11*	ARN	Profilin-like protein (*T. gondii*) *E. coli*

*: Only functionally expressed in mice.

A number of studies have provided clear evidence that leukocyte-driven inflammatory responses play a key role in various immune inflammatory diseases of the kidney. Exogenous ligands that activate TLR3, which has been shown to be expressed in glomerular cells, and TLR7 and TLR9, which are expressed in circulating leukocytes but not in renal epithelial cells, have been shown to exacerbate glomerular lesions in murine systemic lupus erythematosus.[42-44] Using reciprocal chimeric mice, Robson's group showed that in addition to neutrophils,[45,46] intrinsic renal glomerular and mesangial cells are also involved in TLR2- and TLR4-ligand activated, immune-mediated glomerulonephritis.

TLR4 plays a central role in inducing the innate immune response during ascending UTI and APN. The mechanisms underlying the TLR-mediated inflammatory responses have been extensively studied in experimental models of ascending UTI, using transurethral inoculations of uropathogenic strains of *E. coli*. Pioneer studies have identified a point muta-

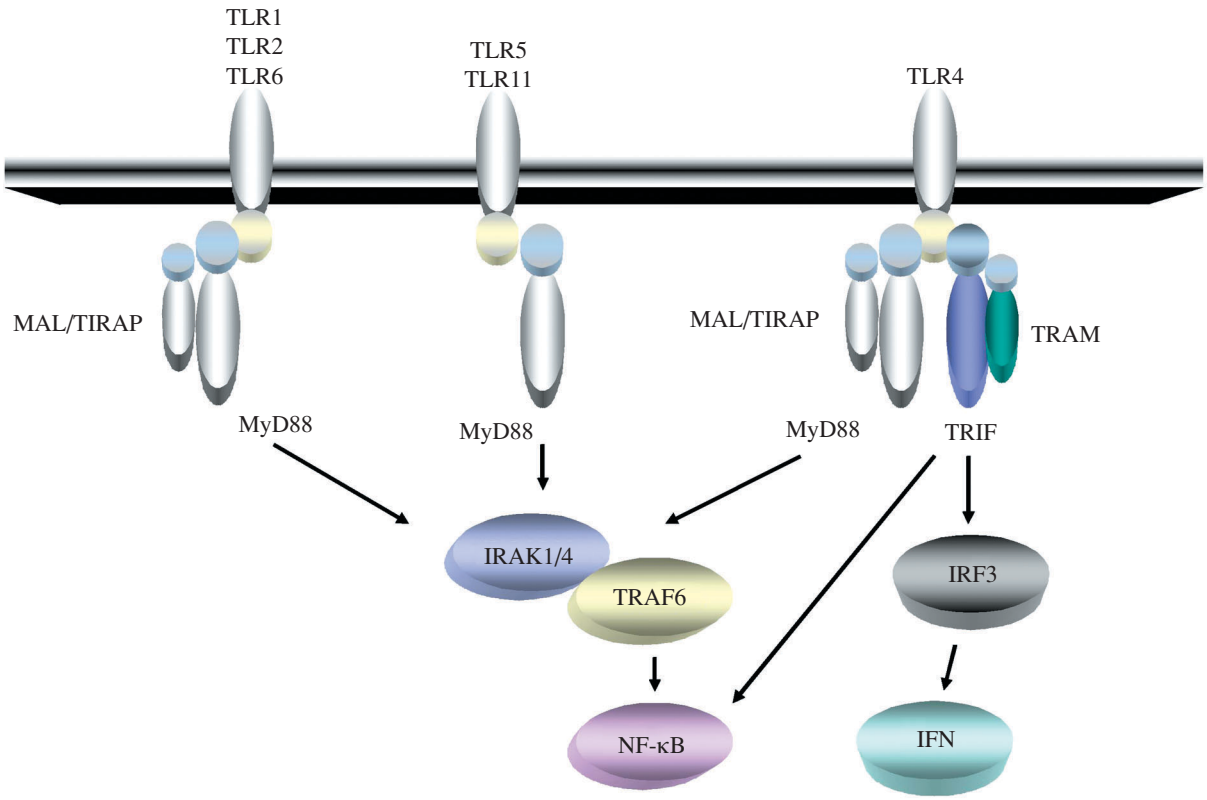

Fig. 1 Diagram depicting the TLR signaling pathways expressed in renal epithelial cells. TLR1, 2, 4, 5, 6 and 11 use different adaptor signaling molecules to regulate the activation of NF-κB, interferon regulatory factor 3 (IRF3), interferon (IFN), and MAP kinases (not shown in the figure).

tion in the *TLR4* gene in the LPS-defective C3H/HeJ mouse strain, and a null-mutation on the *TLR4* gene in LPShyporesponsive C57BL10/ScCr mice.[21,47] Mice rendered TLR4 deficient by targeted disruption of the *TLR4* gene exhibit the same LPS-hyporesponsiveness phenotype, confirming that TLR4 is indeed the receptor for LPS.[48] TLR4 mutations linked to LPS hyporesponsiveness have been also identified in humans.[49] Analysis of the inflammatory response in the bladder and kidneys of irradiated wild-type and TLR4-deficient mice reconstituted with either TLR4-deficient or wild-type bone-marrow derived cells, respectively, has shown that, in addition to bone-marrow cells, intrinsic mucosal cells are also actively involved in inducing the inflammatory response.[50,51] Similar findings have been also reported in models of intestinal mucosal inflammation. Several enterocyte cell lines, and primary and cultured colon cells express TLR4, the adaptors MD2 and MyD88, and cell activation caused by LPS leads to the stimulation of pro-inflammatory mediators.[52-56] Cellular TLR4 has been reported to play a critical role in the pathogenesis of necrotizing enterocolitis.[57] Moreover, activation of TLR4 signaling has been shown to promote the internalization and phagocytosis of *E. coli*,[58] suggesting that activation of TLR4 signaling is involved in the internalization and translocation of Gram-negative bacteria across the intestinal epithelium.

One of the main questions remaining to be answered is whether epithelial cells, which can trigger TLR-mediated pro-inflammatory responses, are in fact primary movers in the innate immune response. Under normal conditions, the mucosal surfaces of the intestine and lung are constantly exposed to environmental commensal bacteria. In contrast, the epithelial cells of the bladder and kidney do not usually come into contact with pathogens. The cur-

rent view is that leukocytes control and coordinate the immune response during inflammatory processes. However, since epithelial cells express functional TLRs, they could also play a role in inducing inflammatory responses, at least under certain specific circumstances. Gribar et al. have proposed two possible mechanisms for the immune response in inflamed tissues, one of which is leukocyte driven and the other, epithelial cell driven.[19] Fig. 2 is a diagram depicting these two hypothetical mechanisms in inflamed kidneys.

Renal tubule epithelial cells act as sensors of the innate immune response triggered by pyelonephritis-associated uropathogenic *E. coli*

Bacterial attachment to mucosal epithelial cells by fimbrial adhesins is the initial step in the pathogenicity of uropathogenic *E. coli* (UPEC).[59] The binding of adhesins to epithelial cell receptors results in tissue specificity, and allows UPECs to ascend into the lower urinary tract and the kidney. Pyelonephritis-associated UPEC strains usually express type-1 fimbriae, which bind to mannosylated glycoprotein, which is abundant in the lower urinary tract.[60,61] They also express P-fimbriae, which bind to the glycosphingolipids that are abundant on the surface of renal epithelial cells.[62,63]

Epithelial TLR4 and ascending urinary tract infections

The attachment of *E. coli* to bladder epithelial cells induces a potent TLR4-mediated inflammatory response.[64] Using an experimental model of ascending UTI in TLR4-expressing and TLR4-defective C3H/HeJ mice, Chassin et al. have shown that UPEC strains colonizing kidneys preferentially adhere to the apical surface of collecting duct tubules,[34] which consist mainly of intercalated cells. Analysis of pri-

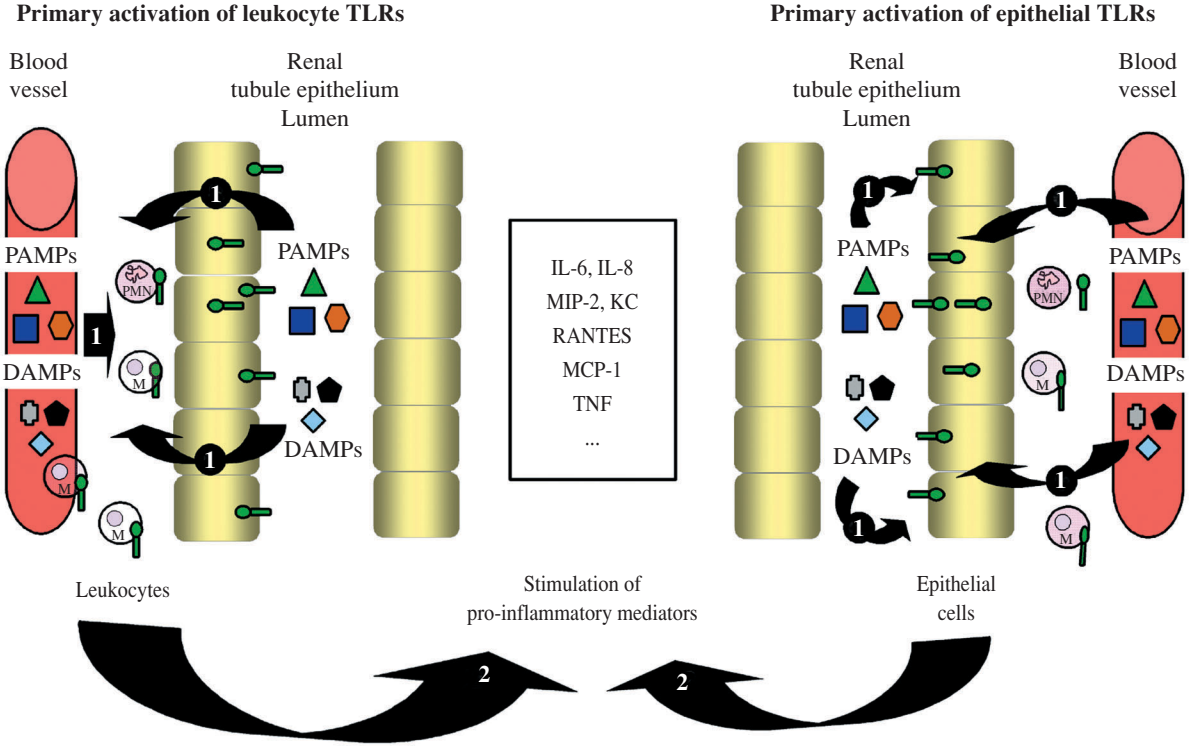

Fig. 2 Dual mechanisms of TLR activation leading to mucosal inflammation. (Left panel) TLR signaling is primarily activated in circulating leukocytes by pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs). (Right panel) PAMPs and DAMPs first activate TLR signaling in epithelial cells. These two mechanisms are not mutually exclusive, and in both cases lead to the secretion of pro-inflammatory mediators, which in turn favor the recruitment of neutrophils, macrophages, and lymphocytes to the site of inflammation. (Redrawn from ref 19.)

mary cultures of medullary collecting ducts dissected from the kidneys of C3H/HeOuJ mice and C3H/HeJ mice has revealed that UPECs predominantly activate TLR4-MyD88-dependent signaling, and also a TLR4-independent signaling pathway involving TNF-receptor-associated factor (TRAF2), pro-apoptotic apoptosis signal-regulatory kinase protein (ASK1), and c-Jun N-terminal kinase (JNK).[33] It has also been shown that the binding of type-1 fimbriae and P-fimbriae to their respective receptors activates TLR4 signaling through the recruitment of different adaptors, suggesting that, independently of LPS, fimbrial adhesins may activate TLR4-dependent signaling pathway(s).[65] This implies that the susceptibility to UTI is controlled by the innate immune response, and that Toll- like receptors, particularly TLR4, are the sentinels of this response. When activated, TLR4 signaling may initiate the symptomatic disease process, while in the absence of TLR4 signaling, the infected host may instead develop an asymptomatic carrier state.

Hormonal control of epithelial TLR4 by arginine vasopressin

Renal collecting tubule cells play a key role in the reabsorption of ions and water, and are controlled by corticosteroid hormones and arginine vasopressin (AVP). We have shown that deamino-arginine vasopressin (dDAVP) acts as a potent inhibitor of the cellular activation induced by LPS in cultured collecting duct cells by a mechanism requiring the serine/threonine protein phosphatase 2A (PP2A) and the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR).[66] *In vivo* administration of dDAVP also increases renal bacterial colonization of UPECs by inhibiting the TLR4-mediated inflammatory response. Moreover, administration of SR121463B, a potent nonpeptide V2 receptor antagonist (also known as Vaptan) of dDAVP, lowers the bacterial loads of infected kidneys by stimulating the local, innate, intrarenal response elicited by collecting duct cells and by its aquaretic effects. These findings have provided a molecular basis for the current advice that patients should be hyperhydrated, which suppresses AVP levels and increases urine flow, in the context of both UTI and APN. Further studies will be necessary to find out whether the administration of V2 receptor antagonists, along with antibiotics, is effective in preventing recurrent UTI and APN, particularly in patients exhibiting elevated levels of circulating AVP.[67]

Epithelial TLR4-mediated internalization and translocation of uropathogenic E. coli

Although the process of UPEC invasion of bladder epithelial cells has been extensively studied,[68-70] the mechanisms that govern the translocation of bacteria from the renal tubular lumen into the renal interstitium, ultimately leading to systemic sepsis, remain less well known. The invasion of bladder epithelial cells by type-1 fimbriated *E. coli* requires caveolin-1,[71] which is highly expressed in the caveolae, a sub-domain of the biochemically defined lipid raft.[72,73] Lipid rafts are used by various pathogens to gain entry into cells. The integrity of lipid rafts has been shown to be necessary for LPS-induced cell activation.[74,75] Using non-pathogenic and pathogenic strains, we identified two modes of cellular translocation of UPECs across the renal tubule epithelial barrier.[76] The UPEC strain CFT073, which expresses cytolytic and vacuolating cytotoxins, disrupted the integrity of cell layers, whereas non-cytolytic UPEC strains translocated through intact collecting duct cell layers without altering the tight junctions. The apical-to-basal transcellular translocation of UPECs was dramatically reduced after the extinction of TLR4 and the lipid raft marker caveolin-1 by small interfering RNAs. Disruption of the lipid rafts by cholesterol-affecting drugs significantly reduced both LPS-induced cell activation and the transcellular translocation of UPECs across the cell layers. Fig. 3 summarises the two possible mechanisms of translocation of UPECs across tight epithelial collecting duct cells. These findings constitute the first demonstration that the transcellular translocation of UPECs across impermeant medullary collecting duct cell layers may occur through lipid rafts *via* a TLR4-facilitated process.[76]

The role of epithelial TLR4 in mediating drug-induced nephrotoxicity

Cisplatin is a potent cancer chemotherapy drug that can induce acute renal failure.[77] Cisplatin and LPS induce synergistic, TLR4-mediated, renal toxicity.[78] Cisplatin-treated, wild-type mice exhibit greater renal cell damage than their cisplatin-treated, TLR4 deficent mouse counterparts.[79] Using a bone-marrow chimeric approach, Zhang et al. have shown

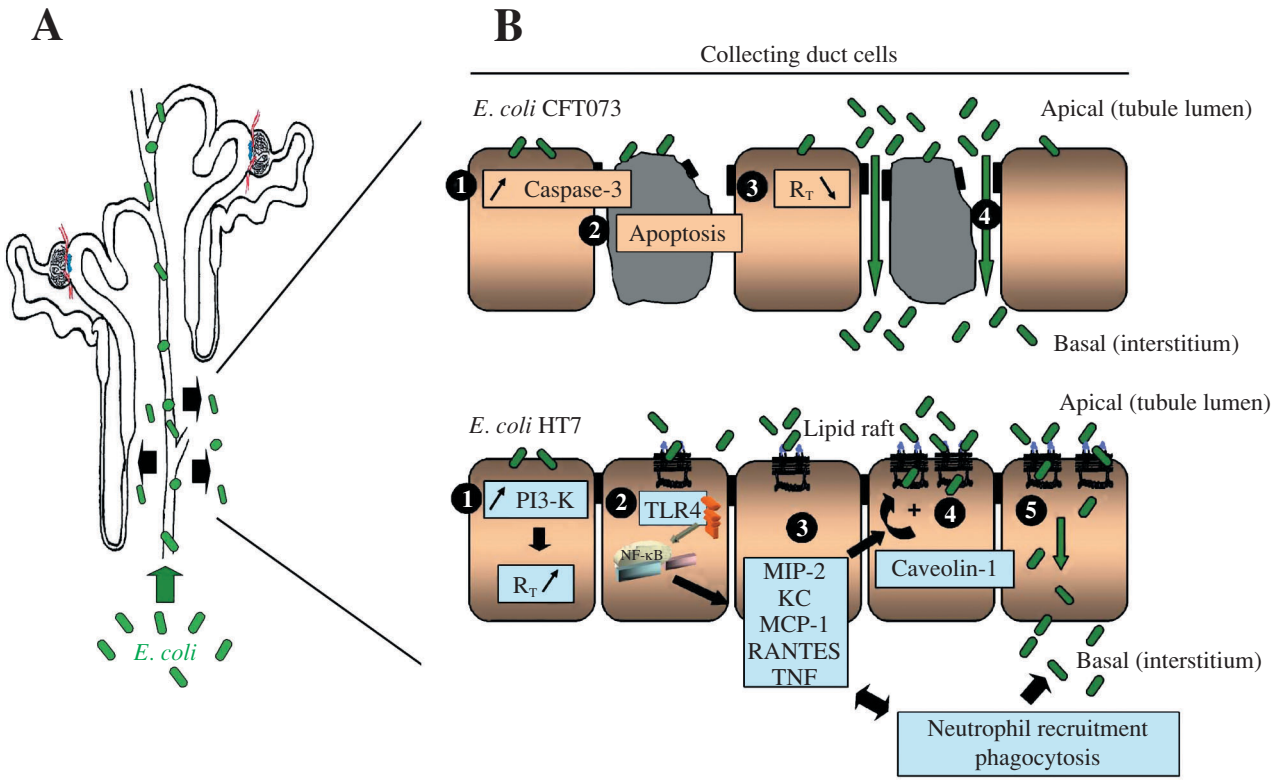

Fig. 3 Translocation of UPECs across the renal tubule epithelium. (A) Uropathogenic *E. coli* adhering to collecting duct cells during their retrograde ascent can invade (arrows) the renal interstitium *via* two distinct mechanisms. (B, upper panel) UPEC strain CFT073 expressing cytolytic α-hemolysin and vacuolating secreted autotransporter toxin (SAT) can disrupt the integrity of confluent layers of collecting tubule cells grown on filters *via* the activation of caspase 3 (1), leading to apoptosis of epithelial cells (2). This in turn is associated with the disruption of tight junctions, characterized by a fall in transepithelial electrical resistance (R_T, 3), leading to the massive translocation of bacteria (4) from the apical side (i.e. the tubule lumen) to the basal side (i.e. the interstitium) of the cell layers. (B, lower panel) The non-cytolytic UPEC strain HT7 induces the activation of PI3-kinase (PI3-K), and a rise in R_T (1) reflecting the intracellular redistribution of cytoskeletal elements. Adhesion of UPECs to the apical surface of collecting duct cells induces TLR4-mediated activation of the transcription factor NF-κB (2), and the subsequent production of pro-inflammatory cytokines (3), leading to the recruitment of neutrophils for efficient phagocytosis and the killing of bacteria invading the renal interstitium. TLR4-mediated cell activation is associated with membrane recruitment of caveolin-1 into lipid rafts (4), which favors the apical-to-basal translocation of bacteria. From ref 76.

that parenchymatous mucosal TLR4,[79] rather than bone-marrow derived cells, mediates the activation of the p38 MAP kinase pathway, leading to increased production of cytokines and subsequent kidney injury.

Role of antimicrobial peptides and proteins in the defense of renal epithelial cells against uropathogenic bacteria

Under normal conditions, the urinary tract, unlike the intestine and lungs, constitutes a sterile environment. The epithelial cells lining the urinary tract system produce and secrete a number of antimicrobial peptides (AMPs) and proteins, which help to maintain the sterility of the urine through their antimicrobial action.[80]

Defensin and cathelicidin

Defensins and cathelicidin (also known as LL37) are the AMPs that have undergone the most extensive investigation. The α-defensins HNP1-4 are expressed in neutrophils,[81] and β-defensins HD5 and HD6 are expressed in Paneth cells located in the crypts of the small intestine.[82] No there is normally

no spaces betwee, HNP1-4. Cathelicidin is widely expressed in leukocytes and epithelial cells. Both defensins and cathelicidin are expressed in the urinary tract and epididymis,[83,84] and display rapid antimicrobial effects that preserve the sterility of urine. In contrast to the multiple defensins, a single gene, *CAMP* (for cathelicidin antimicrobial peptide), encodes cathelicidin.[85-87] Epithelial cells expressing cathelicidin have been shown to contribute to protecting the urinary tract, since bacterial loads from *E. coli* colonizing kidneys are greater in infected, cathelicidin-deficient mice than in their infected, wild-type counterparts.[88]

Tamm-Horsfall protein

The Tamm-Horsfall protein (THP or uromucoid), which is only expressed and secreted by the thick ascending limb cells, is the most abundant kidney-specific glycoprotein found in the urine.[89] THP, which stimulates pro-inflammatory mediators in human monocytes and granulocytes,[90,91] activates dendritic cells *via* a TLR4-dependent mechanism,[92] suggesting that THP has an immunoregulating effect in the context of urinary tract infections (UTIs). Interestingly, bacterial loads of fimbriated *E. coli* were higher in the bladder, but not in the kidneys of THP-deficient mice compared to controls, suggesting that THP may impair the attachment of *E. coli* to uroepithelial cells.[93] THP was also shown to protect kidneys against ischemic injury by reducing the inflammatory response, possibly through TLR4-mediated signaling.[94]

Lipocalin, lactoferrin

Lipocalin and lactoferrin display antimicrobial activity due to their ability to chelate iron, which is an essential bacterial nutrient. Lactoferrin is expressed in distal and collecting-duct epithelial cells.[95] Lipocalin-deficient mice were also shown to be more susceptible to systemic *E. coli* infection, but not to I/R injury.[96] Overall, these studies have provided evidence that, *via* their antimicrobial actions, AMP and other proteins secreted by renal tubule cells help to eliminate bacteria from the urinary tract.

A role for epithelial TLR2 and TLR4 in the renal inflammation and cell damage caused by ischemia-reperfusion injury

Activation of the innate immune system can also be initiated by trauma or ischemia. Such non-bacterial, "sterile" inflammatory responses result from the capacity of DAMPs to activate immune signaling pathways through common pattern recognition receptors. DAMPs can be classified as endogenous constituents released by damaged or necrotic cells, and as components of the extracellular matrix that are released by proteases. Table 2 summarizes the main DAMP constituents that can activate TLR signaling.[97,98] They include the high-mobility group box 1 (HMGB1),[99-101] heat shock proteins,[102-104] fibrinogen,[105] fibronectin,[106] surfactant,[107] β-defensin,[108] uric acid,[109] hyaluronan,[110] and the matrix components biglycan and heparan sulfate.[111,112] Most of them have been shown to activate TLR2 and TLR4. Genomic DNA from dying cells also induces the maturation of antigen-presenting cells,[113] and self-mRNA released from necrotic cells activates TLR3.[114] HMGB1, which is ubiquitously expressed in eukaryotic cells, is a member of the non-histone, chromatin-associated, high-mobility group (HMG) family of proteins.[115] HMGB1, one of the DAMPs that has undergone the most extensive investigation, is released by all cells in a context of necrotic cell death.[116] It can also be secreted by macrophages or dendritic cells in response to LPS, interferon-γ, and TNF-α.[117] Like TLR2, TLR4 recognizes a wide range of DAMPs (Table 2). TLR4 has

Table 2. Endogenous DAMP Ligands Activating TLR Signaling

Endogenous DAMP ligands
Heat shock proteins
(Hsp60, Hsp70, Hsp90, gp96)
Fibronectin
Hyaluronic acid
Heparan sulfate
Fibrinogen
Surfactant Protein-A
β-defensins
High mobility group box 1 (HMGB1)
Cell DNA
RNA
Uric acid

been implicated in the mucosal inflammation caused by ischemia/reperfusion (I/R) in hepatic I/R,[118,119] cardiac I/R,[120] and hemorrhagic shock.[121,122] TLR4 and TLR2 also mediate inflammatory responses during I/R injury.[123,124] The expression levels of both TLR2 and TLR4 increase rapidly in post-ischemic renal tubule epithelial cells (RTECs).[125] Conversely, TLR2- and TLR4-deficient mice are better protected than wild-type mice.[123,124,126] MyD88 deficient mice are also better protected against I/R injury than wild-type mice. Experiments performed on primary cultures of RTECs also revealed that TLR2 and TLR4 drive the inflammatory response and apoptosis of renal RTECs.[123,124] These findings suggest that TLR2 and TLR4 are activated by endogenous ligands released by damaged cells to engage both MyD88-dependent and MyD88-independent signaling pathways.[124,126] The mechanisms of the TLR-mediated downstream signaling activated during I/R are, however, less well known. Recently, Tsung et al. showed that HMGB1 released from cultured hepatocytes required intact TLR4,[118] and was regulated by reactive oxygen species (ROS). The release of HMGB1 by oxidative stress was reduced by inhibiting the calcium/calmodulin-dependent kinases (CMKs) involved in calcium-dependent signaling pathways.[127] The oxidative stress injury caused by I/R injury has been attributed to the activation of MAP kinase pathways, including those of extracellular signal-regulated protein kinase (ERK), JNK and p-38.[128,129] Activation of JNK/p38, via the activation of the Ser/Thr MAP kinase kinase, apoptosis signal-regulating kinase 1 (ASK1), plays a key role in cytokine- and stress-induced apoptosis.[130,131] The activation of ERK has been shown to be associated with drug-induced apoptosis,[132-134] but has also been implicated in cell survival following oxidant injury or the induction of endoplasmic reticulum (ER), stress-induced, cell death signaling.[135-137]

Selective TLR2-mediated activation of ERK in ischemic renal tubule epithelial cells

TLR2 and TLR4 both activate JNK, but the activation of ERK1/2 in post-ischemic kidneys appears to be selectively mediated by TLR2.[138] Protein phosphatase 5 (PP5) has been identified as an inactivator of the MEK-ERK pathway through its interaction with the kinase Raf-1, which initiates this pathway.[139] Co-immunoprecipitation studies have shown that PP5 forms a complex with gp96, the ER-resident homologue of cytosolic Hsp90, which is known to play a key role in TLR-mediated, innate immunity,[140,141] in non-hypoxic, wild-type RTECs. PP5 no longer co-immunoprecipitates with gp96 in post-hypoxic, wild-type mice kidneys. In contrast, PP5 still co-immunoprecipitates with gp96 in protected, post-hypoxic, TLR2-deficient RTECs. These findings led to the suggestion that it is the disruption of the gp96-PP5 interaction that leads to the inactivation of PP5. Furthermore, extinction of PP5 mRNA expression by silencing the RNA restores both the activation of ERK1/2, and ASK1-, JNK-mediated cell apoptosis in TLR2-deficient RTECs. Fig. 4 shows a diagram of the mechanism of the TLR2-mediated activation of ERK1/2 in RTECs subjected to transient hypoxia.

Contribution of epithelial donor TLR4s to acute rejection and ischemia/reperfusion injury in renal grafts

The extent to which TLR-dependent innate immune mechanisms contribute to the rejection of renal grafts is still debated. Transplant studies using C3H/HeOuJ and TLR4-defective C3H/HeJ mice have revealed that wild-type recipients of C3H/HeJ kidneys develop severe acute renal failure (ARF), whereas C3H/HeJ recipients of wild-type kidneys were protected from LPS-induced ARF,[142] suggesting that both epithelial cells and hematopoietic cells are involved in the complex mechanism of endotoxin-induced ARF. Two single mutations (Asp299Gly and The399Ile) in the *TLR4* gene associated with endotoxin hyporesponsiveness have been identified in humans.[49] Conflicting results in the context of renal grafts have been reported regarding the consequences of the expression of these two mutated TLR4s on the incidence of acute rejection.[143-146] The expression of TLR4 and MyD88 in peripheral blood mononuclear cells and in graft biopsies is elevated in chronic rejection patients, suggesting that there may be a link between TLR4 expression and long-term graft outcome.[146] Recently, Krüger et al. have shown that the TLR4 ligand HMGB1,[147] which is undetectable in kidneys from living donors, is expressed in epithelial cells from tubule sections of kidneys grafts taken from deceased donor patients. Moreover, renal grafts expressing both the TLR4 Asp299Gly and TLR4 Thre399Ile alleles were better protected than those expressing wild-type alleles. This study

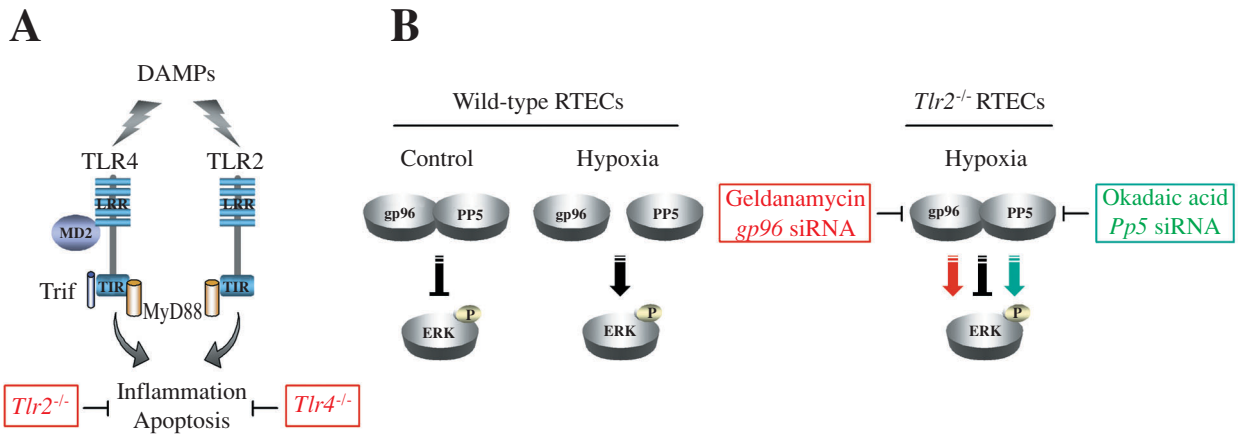

Fig. 4 Involvement of TLR2 and TLR4 in mediating the inflammatory response and apoptosis during renal ischemia/reperfusion injury. (A) Diagram depicting the activation of TLR2 and TLR4 in kidneys subjected to ischemia/reperfusion injury. (B) TLR2-mediated ERK activation in post-hypoxic renal tubule epithelial cells. (Left panel) Protein phosphatase 5 (PP5) is associated with gp96 in resting (Control), wild-type, renal tubule epithelial cells (RTECs). (Middle panel) Hypoxia induces the dissociation of gp96 bound to PP5 in wild-type RTECS, resulting in the inhibition of PP5 activity, and downstream ERK1/2 phosphorylation. (Right panel) TLR2 deficient (*Tlr2*$^{-/-}$) RTECs are protected against hypoxia, and do not provoke the dissociation of gp96 bound to PP5. In this case, PP5 remains active, and inhibits downstream ERK1/2 phosphorylation. Inhibiting the activity of gp96 by geldanamycin or silencing *gp96* mRNA expression (in red) induces the reactivation of ERK1/2. Inhibition of PP5 activity by okadaic acid or silencing *Pp5* mRNA expression (in green) also induces the reactivation of ERK1/2 phosphorylation and of p-JNK and ASK-1 (not shown). (From ref 138.)

has provided strong evidence that epithelial cells from kidney donors producing TLR4 and HMGB1 contribute to I/R injury following kidney transplantation.

Conclusions and future perspectives

There is now growing evidence that epithelial renal tubule cells are actively involved in the onset of an innate immune response to both ascending urinary tract infections and renal I/R injury. Both TLR2 and TLR4 in the case of I/R injury, and TLR4 in the case of ascending UTI and APN caused by Gram-negative bacteria, play a key role in initiating a potent inflammatory defense response by the host. Many of the studies discussed in this review suggest that there is close interplay between activated leukocytes and epithelial cells. Although TLR4 plays a major role in triggering an innate immune response in the course of ascending UTI, we cannot rule out the possibility that other innate receptors expressed by renal epithelial cells may also be involved in triggering the innate immune response. These include TLR5, which recognizes flagellin, and seems to be involved in the innate immune responses elicited by flagellated bacteria. Flagellum-mediated motility allows flagellin-expressing UPECs to ascend the lower urinary tract and invade the kidneys.[148-150] Using a set of wild-type UPEC strains and mutants that lack flagellin or the motor proteins MotA and MotB, Pichon et al. reported that the motility of one invasive UPEC strain appears to be required to promote contact with renal collecting duct cells,[151] which are the preferred intrarenal target cells for UPECs,[34] and the subsequent cellular internalization of the UPECs. In addition, Andersen -Nissen has reported that TLR5-deficient mice exhibited a less intense inflammatory response and greater bacterial loads in the bladder than their infected,[39] wild-type counterparts two days after transurethral inoculation with the CFT073 UPEC strain. Similarly, bacterial loads were greater in the infected kidneys of TLR5-deficient mice 5 days after the bacteria were inoculated.[39] Taken together these studies provide evidence that TLR5 is involved in controling the inflammatory response, cell internalization, and cell invasion during UTI and APN. A recent study has also revealed that a TLR5-polymorphism (Ala896Gly), which encodes a variant that abrogates

flagellin-induced signaling, is associated with an increased risk of UTI.[152]

Identification of the proteins involved in controling the activated, TLR-mediated signaling pathways certainly offers an interesting approach to the development of novel pharmacological strategies to reduce inflammatory responses and prevent damage to renal cells. Preventing cell damage and reducing the inflammatory responses that occur during the reimplantation of cold-stored cadaveric kidneys grafts can be expected to reduce the incidence of delayed graft function, and, hopefully, lead to better preservation of long-term graft function.

Acknowledgements

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM) and in part by a INSERM/NCS travel grant, and grants from the Agence Nationale de la Recherche (ANR-08-MIEN-030-01) and Agence de la Biomédecine (Recherche et Greffe R0709HH, 2008). A. Vandewalle received an Interface INSERM-APHP fellowship.

REFERENCES

1. Brown P, Ki M, Foxman B. Acute pyelonephritis among adults: cost of illness and considerations for the economic evaluation of therapy. Pharmacoeconomics 2005;23:1123-42.
2. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol 2005;295:383-404.
3. Freedman AL. Urologic diseases in North America Project: trends in resource utilization for urinary tract infections in children. J Urol 2005;173:949-54.
4. Abbott KC, Swanson SJ, Richter ER, Bohen EM, Agodoa LY, Peters TG, Barbour G, Lipnick R, Cruess DF. Late urinary tract infection after renal transplantation in the United States. Am J Kidney Dis 2004;44:353-62.
5. Pellé G, Vimont S, Levy PP, Hertig A, Ouali N, Chassin C, Arlet G, Rondeau E, Vandewalle A. Acute pyelonephritis represents a risk factor impairing long-term kidney graft function. Am J Transplant 2007;7:899-907.
6. de Souza RM, Olsburgh J. Urinary tract infection in the renal transplant patient. Nat Clin Pract Nephrol 2008;4:252-64.
7. Säemann M, Hörl WH. Urinary tract infection in renal transplant recipients. Eur J Clin Invest 2008;38 Suppl 2:58-65.
8. Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J Antimicrob Chemother 2008;61:1024-8.
9. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. Science 2003;301:105-7.
10. Justice SS, Hunstad DA, Seed PC, Hultgren SJ. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. Proc Natl Acad Sci USA 2006;103:19884-9.
11. Wright KJ, Seed PC, Hultgren SJ. Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. Cell Microbiol 2007;9:2230-41.
12. Kamath NS, John GT, Neelakantan N, Kirubakaran MG, Jacob CK. Acute graft pyelonephritis following renal transplantation. Transpl Infect Dis 2006;8:140-7.
13. Gribar SC, Anand RJ, Sodhi CP, Hackam DJ. The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. J Leukoc Biol 2008;83:493-8.
14. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499-511.
15. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 2007;7:353-64.
16. Werts C, Girardin SE, Philpott DJ. TIR, CARD and PYRIN: three domains for an antimicrobial triad. Cell Death Differ 2006;13:798-815.
17. Franchi L, Warner N, Viani K, Nuñez G. Function of Nod-like receptors in microbial recognition and host defense. Immunol Rev 2009;227:106-28.
18. Ip WK, Takahashi K, Ezekowitz RA, Stuart LM. Mannose-binding lectin and innate immunity. Immunol Rev 2009;230:9-21.
19. Gribar SC, Richardson WM, Sodhi CP, Hackam DJ. No longer an innocent bystander: epithelial toll-like receptor signaling in the development of mucosal inflammation. Mol Med 2008;14:645-59.
20. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1-5.
21. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998;282:2085-8.
22. Baeuerle PA, Baltimore D. I kappa B: a specific inhibitor of the NF-kappa B transcription factor. Science 1988;242:540-6.
23. Brown K, Gerstberger S, Carlson L, Franzoso G, Siebenlist U. Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. Science 1995;267:1485-8.
24. Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao

Z, Matsumoto K. The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. Nature 1999;398:252-6.
25. An H, Yu Y, Zhang M, Xu H, Qi R, Yan X, Liu S, Wang W, Guo Z, Guo J, Qin Z, Cao X. Involvement of ERK, p38 and NF-kappaB signal transduction in regulation of TLR2, TLR4 and TLR9 gene expression induced by lipopolysaccharide in mouse dendritic cells. Immunology 2002;106:38-45.
26. Schieven GL. The biology of p38 kinase: a central role in inflammation. Curr Top Med Chem 2005;5:921-8.
27. Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim SO, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J, Beutler B. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 2003;424:743-8.
28. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okae M, Takeda M, Akira S. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. Science 2003;301:640-3.
29. Vandewalle A. Toll-like receptors and renal bacterial infections. Chang Gung Med J 2008;31:525-37.
30. Tsuboi N, Yoshikai Y, Matsuo S, Kikuchi T, Iwami K, Nagai Y, Takeuchi O, Akira S, Matsuguchi T. Roles of toll-like receptors in C-C chemokine production by renal tubular epithelial cells. J Immunol 2002;169:2026-33.
31. Wolfs TG, Buurman WA, van Schadewijk A, de Vries B, Daemen MA, Hiemstra PS, van 't Veer C. In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-gamma and TNF-alpha mediated up-regulation during inflammation. J Immunol 2002;168:1286-93.
32. Yang CW, Hung CC, Wu MS, Tian YC, Chang CT, Pan MJ, Vandewalle A. Toll-like receptor 2 mediates early inflammation by leptospiral outer membrane proteins in proximal tubule cells. Kidney Int 2006;69:815-22.
33. Chowdhury P, Sacks SH, Sheerin NS. Toll-like receptors TLR2 and TLR4 initiate the innate immune response of the renal tubular epithelium to bacterial products. Clin Exp Immunol 2006;145:346-56.
34. Chassin C, Goujon JM, Darche S, du Merle L, Bens M, Cluzeaud F, Werts C, Ogier-Denis E, Le Bouguénec C, Buzoni-Gatel D, Vandewalle A. Renal collecting duct epithelial cells react to pyelonephritis-associated Escherichia coli by activating distinct TLR4-dependent and -independent inflammatory pathways. J Immunol 2006;177:4773-84.
35. Shigeoka AA, Holscher TD, King AJ, Hall FW, Kiosses WB, Tobias PS, Mackman N, McKay DB. TLR2 is constitutively expressed within the kidney and participates in ischemic renal injury through both MyD88-dependent and -independent pathways. J Immunol 2007;178:6252-8.
36. El-Achkar TM, Huang X, Plotkin Z, Sandoval RM, Rhodes GJ, Dagher PC. Sepsis induces changes in the expression and distribution of Toll-like receptor 4 in the rat kidney. Am J Physiol Renal Physiol 2006;290:F1034-43.
37. Bäckhed F, Söderhäll M, Ekman P, Normark S, Richter-Dahlfors A. Induction of innate immune responses by Escherichia coli and purified lipopolysaccharide correlate with organ- and cell-specific expression of Toll-like receptors within the human urinary tract. Cell Microbiol 2001;3:153-8.
38. Samuelsson P, Hang L, Wullt B, Irjala H, Svandborg C. Toll-like receptor 4 expression and cytokine responses in the human urinary tract mucosa. Infect Immun 2004;72:3179-86.
39. 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;178:4717-20.
40. 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;303:1522-6.
41. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, Sher A. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 2005;308:1626-9.
42. Patole PS, Gröne HJ, Segerer S, Ciubar R, Belemezova E, Henger A, Kretzler M, Schlöndorff D, Anders HJ. Viral double-stranded RNA aggravates lupus nephritis through Toll-like receptor 3 on glomerular mesangial cells and antigen-presenting cells. J Am Soc Nephrol 2005;16:1326-38.
43. Anders HJ, Vielhauer V, Eis V, Linde Y, Kretzler M, Perez de Lema G, Strutz F, Bauer S, Rutz M, Wagner H, Gröne HJ, Schlöndorff D. Activation of toll-like receptor-9 induces progression of renal disease in MRL-Fas(lpr) mice. FASEB J 2004;18:534-6.
44. Pawar RD, Patole PS, Zecher D, Segerer S, Kretzler M, Schlöndorff D, Anders HJ. Toll-like receptor-7 modulates immune complex glomerulonephritis. J Am Soc Nephrol 2006;17:141-9.
45. Giorgini A, Brown HJ, Lock HR, Nimmerjahn F, Ravetch JV, Verbeek JS, Sacks SH, Robson MG. Fc gamma RIII and Fc gamma RIV are indispensable for acute glomerular inflammation induced by switch variant monoclonal antibodies. J Immunol 2008;181:8745-52.
46. Brown HJ, Lock HR, Wolfs TG, Buurman WA, Sacks SH, Robson MG. Toll-like receptor 4 ligation on intrinsic renal cells contributes to the induction of antibody-mediated glomerulonephritis via CXCL1 and CXCL2. J Am Soc Nephrol 2007;18:1732-9.
47. Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). J Exp Med 1999;189:615-25.
48. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T,

Takeda Y, Takeda K, Akira S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol 1999;162:3749-52.
49. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000;25:187-91.
50. Schilling JD, Martin SM, Hung CS, Lorenz RG, Hultgren SJ. Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic *Escherichia coli*. Proc Natl Acad Sci USA 2003;100:4203-8.
51. Patole PS, Schubert S, Hildinger K, Khandoga S, Khandoga A, Segerer S, Henger A, Kretzler M, Werner M, Krombach F, Schlöndorff D, Anders HJ. Toll-like receptor-4: renal cells and bone marrow cells signal for neutrophil recruitment during pyelonephritis. Kidney Int 2005;68:2582-7.
52. Neal MD, Leaphart C, Levy R, Prince J, Billiar TR, Watkins S, Li J, Cetin S, Ford H, Schreiber A, Hackam DJ. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. J Immunol 2006;176:3070-9.
53. Ruemmele FM, Beaulieu JF, Dionne S, Levy E, Seidman EG, Cerf-Bensussan N, Lentze MJ. Lipopolysaccharide modulation of normal enterocyte turnover by toll-like receptors is mediated by endogenously produced tumour necrosis factor alpha. Gut 2002;51:842-8.
54. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000;68:7010-7.
55. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004;126:1054-70.
56. Hornef MW, Frisan T, Vandewalle A, Normark S, Richter-Dahlfors A. Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. J Exp Med 2002;195:559-70.
57. Jilling T, Simon D, Lu J, Meng FJ, Li D, Schy R, Thomson RB, Soliman A, Arditi M, Caplan MS. The roles of bacteria and TLR4 in rat and murine models of necrotizing enterocolitis. J Immunol 2006;177:3273-82.
58. Neal MD, Leaphart C, Levy R, Prince J, Billiar TR, Watkins S, Li J, Cetin S, Ford H, Schreiber A, Hackam DJ. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. J Immunol 2006;176:3070-9.
59. Bergsten G, Wullt B, Svanborg C. *Escherichia coli*, fimbriae, bacterial persistence and host response induction in the human urinary tract. Int J Med Microb 2005;295:487-502.
60. Connell I, Agace W, Klemm P, Schembri M, Marild S, Svanborg C. Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proc Natl Acad Sci USA 1996;93:9827-32.
61. Leffler H, Svanborg-Edén C. Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol Lett 1980;8:117-82.
62. Roberts JA, Marklund BI, Ilver D, Hasla D, Kaack MB, Baskin G, Louis M, Mollby R, Winberg J, Normark S. The Gal(alpha 1-4)Gal-specific tip adhesin of *Escherichia coli* P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc Natl Acad Sci USA 1994;91:11889-93.
63. Nowicki B, Moulds J, Hull R, Hull S. A hemagglutinin of uropathogenic *Escherichia coli* recognizes the Dr blood group antigen. Infect Immun 1988;56:1057-60.
64. Schilling JD, Mulvey MA, Vincent CD, Lorenz RG, Hultgren SJ. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. J Immunol 2001;166:1148-55.
65. Fischer H, Yamamoto M, Akira S, Beutler B, Svanborg C. Mechanism of pathogen-specific TLR4 activation in the mucosa: fimbriae, recognition receptors and adaptor protein selection. Eur J Immunol 2006;36:267-77.
66. Chassin C, Hornef MW, Bens M, Lotz M, Goujon JM, Vimont S, Arlet G, Hertig A, Rondeau E, Vandewalle A. Hormonal control of the renal immune response and antibacterial host defense by arginine vasopressin. J Exp Med 2007;204:2837-52.
67. Knepper MA, Star RA. Vasopressin: friend or foe? Nat Med 2008;14:14-6.
68. Martinez JJ, Mulvey MA, Schilling JD, Pinkne JS, Hultgren SJ. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. EMBO J 2000;19:2803-12.
69. Schilling JD, Mulvey MA, Vincent CD, Lorenz RG, Hultgren SJ. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. J Immunol 2001;166:1148-55.
70. Wullt B, Bergsten G, Connell H, Rollano P, Gebratsedik N, Hang L, Svanborg C. P-fimbriae trigger mucosal responses to *Escherichia coli* in the human urinary tract. Cell Microbiol 2001;3:255-64.
71. Duncan MJ, Li G, Shin JS, Carson JL, Abraham SN. Bacterial penetration of bladder epithelium through lipid rafts. J Biol Chem 2004;279:18944-51.
72. Anderson RG. The caveolae membrane system. Annu Rev Biochem 1998;67:199-225.
73. Kurzchalia TV, Parton RG. Membrane microdomains and caveolae. Curr Opin Cell Biol 1999;11:424-31.
74. Triantafilou M, Miyake K, Golenbock DT, Triantafilou K. Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-

induced cell activation. J Cell Sci 2002;115:2603-11.
75. Hornef MW, Normark BH, Vandewalle A, Normark S. Intracellular recognition of lipopolysaccharide by toll-like receptor 4 in intestinal epithelial cells. J Exp Med 2003;198:1225-35.
76. Chassin C, Vimont S, Cluzeaud F, Bens M, Goujon JM, Fernandez B, Hertig A, Rondeau E, Arlet G, Hornef MW, Vandewalle A. TLR4 facilitates translocation of bacteria across renal collecting duct cells. J Am Soc Nephrol 2008;19:2364-74.
77. Ries F, Klastersky J. Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. Am J Kidney Dis 1986;13:368-79.
78. Ramesh G, Zhang B, Uematsu S, Akira S, Reeves WB. Endotoxin and cisplatin synergistically induce renal dysfunction and cytokine production in mice. Am J Physiol Renal Physiol 2007;293:F325-32.
79. Zhang B, Ramesh G, Uematsu S, Akira S, Reeves WB. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. J Am Soc Nephrol 2008;19:923-32.
80. Zasloff M. Antimicrobial peptides, innate immunity, and the normally sterile urinary tract. J Am Soc Nephrol 2007;18:2810-6.
81. Rehaume LM, Hancock RE. Neutrophil-derived defensins as modulators of innate immune function. Crit Rev Immunol 2008;28:185-200.
82. Bevins CL. Paneth cell defensins: key effector molecules of innate immunity. Biochem Soc Trans 2006;34:263-6.
83. Donovan KL, Topley N. What are renal defensins defending? Nephron Exp Nephrol 2003;93:e125-8.
84. Malm J, Sørensen O, Persson T, Frohm-Nilsson M, Johansson B, Bjartell A, Lilja H, Ståhle-Bäckdahl M, Borregaard N, Egesten A. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. Infect Immun 2000;68:4297-302.
85. Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, Gennaro R. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem 1997;272:13088-93.
86. Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, Borregaard N. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001;97:3951-9.
87. Pestonjamasp VK, Huttner KH, Gallo RL. Processing site and gene structure for the murine antimicrobial peptide CRAMP. Peptides 2001;22:1643-50.
88. Chromek M, Slamová Z, Bergman P, Kovács L, Podracká L, Ehrán I, Hökfelt T, Gudmundsson GH, Gallo RL, Agerberth B, Brauner A. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat Med 2006;12:636-41.
89. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. Am J Kidney Dis 2003;42:658-76.
90. Su SJ, Chang KL, Lin TM, Huang YH, Yeh TM. Uromodulin and Tamm-Horsfall protein induce human monocytes to secrete TNF and express tissue factor. J Immunol 1997;158:3449-56.
91. Kreft B, Jabs WJ, Laskay T, Klinger M, Solbach W, Kumar S, van Zandbergen G. Polarized expression of Tamm-Horsfall protein by renal tubular epithelial cells activates human granulocytes. Infect Immun 2002;70:2650-6.
92. Säemann MD, Weichhart T, Zeyda M, Staffler G, Schunn M, Stuhlmeier KM, Sobanov Y, Stulnig TM, Akira S, von Gabain A, von Ahsen U, Hörl WH, Zlabinger GJ. Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4-dependent mechanism. J Clin Invest 2005;115:468-75.
93. Bates JM, Raffi HM, Prasadan K, Mascarenhas R, Laszik Z, Maeda N, Hultgren SJ, Kumar S. Tamm-Horsfall protein knockout mice are more prone to urinary tract infection: rapid communication. Kidney Int 2004;65:791-7.
94. El-Achkar TM, Wu XR, Rauchman M, McCracken R, Kiefer S, Dagher PC. Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression. Am J Physiol Renal Physiol 2008;295:F534-44.
95. Abrink M, Larsson E, Gobl A, Hellman L. Expression of lactoferrin in the kidney: implications for innate immunity and iron metabolism. Kidney Int 2000;57:2004-10.
96. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, Fong HE, Cheung CC, Mak TW. Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci USA 2006;103:1834-9.
97. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 2009;60:379-406.
98. Srikrishna G, Freeze HH. Endogenous damage-associated molecular pattern molecules at the crossroads of inflammation and cancer. Neoplasia 2009;11:615-28.
99. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 2004;279:7370-7.
100. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005;201:1135-43.
101. Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ, Yang H. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock

2006;26:174-9.
102. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. J Biol Chem 2002;277:15107-12.
103. Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem 2002;277:15028-34.
104. Ohashi K, Burkart V, Flohé S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol 2000;164:558-61.
105. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol 2001;167:2887-94.
106. Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, Chow JC, Strauss JF 3rd. The extra domain A of fibronectin activates Toll-like receptor 4. J Biol Chem 2001;276:10229-33.
107. Guillot L, Balloy V, McCormack FX, Golenbock DT, Chignard M, Si-Tahar M. Cutting edge: the immunostimulatory activity of the lung surfactant protein-A involves Toll-like receptor 4. J Immunol 2002;168:5989-92.
108. Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, Kwak LW. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. Science 2002;298:1025-9.
109. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 2003;425:516-21.
110. Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C, Simon JC. Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. J Exp Med 2002;195:99-111.
111. Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Götte M, Malle E, Schaefer RM, Gröne HJ. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest 2005;115:2223-33.
112. Johnson GB, Brunn GJ, Kodaira Y, Platt JL. Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by Toll-like receptor 4. J Immunol 2002;168:5233-9.
113. Ishii KJ, Suzuki K, Coban C, Takeshita F, Itoh Y, Matoba H, Kohn LD, Klinman DM. Genomic DNA released by dying cells induces the maturation of APCs. J Immunol 2001;167:2602-7.
114. Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. J Biol Chem 2004;279:12542-50.
115. Thomas JO, Travers AA. HMG1 and 2, and related 'architectural' DNA-binding proteins. Trends Biochem Sci 2001;26:167-74.
116. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191-5.
117. Rucci A, Palumbo R, Bianchi ME. HMGB1: a signal of necrosis. Autoimmunity 2007;40:285-9.
118. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005;201:1135-43.
119. Zhai Y, Shen XD, O'Connell R, Gao F, Lassman C, Busuttil RW, Cheng G, Kupiec-Weglinski JW. Cutting edge: TLR4 activation mediates liver ischemia/reperfusion inflammatory response via IFN regulatory factor 3-dependent MyD88-independent pathway. J Immunol 2004;173:7115-9.
120. Oyama J, Blais Jr C, Liu X, Pu M, Kobzik L, Kelly RA, Bourcier T. Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. Circulation 2004;109:784-9.
121. Barsness KA, Arcaroli J, Harken AH, Abraham E, Banerjee A, Reznikov L, McIntyre RC. Hemorrhage-induced acute lung injury is TLR-4 dependent. Am J Physiol Regul Integr Comp Physiol 2004;287:R592-9.
122. Prince JM, Levy RM, Yang R, Mollen KP, Fink MP, Vodovotz Y, Billiar TR. Toll-like receptor-4 signaling mediates hepatic injury and systemic inflammation in hemorrhagic shock. J Am Coll Surg 2006;202:407-17.
123. Leemans JC, Stokman G, Claessen N, Rouschop KM, Teske GJ, Kirschning CJ, Akira S, van der Poll T, Weening JJ, Florquin S. Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. J Clin Invest 2005;115:2894-903.
124. Wu H, Chen G, Wyburn KR, Yin J, Bertolino P, Eris JM, Alexander SI, Sharland AF, Chadban SJ. TLR4 activation mediates kidney ischemia/reperfusion injury. J Clin Invest 2007;117:2847-59.
125. Kim BS, Lim SW, Li C, Kim JS, Sun BK, Ahn KO, Han SW, Kim J, Yang CW. Ischemia-reperfusion injury activates innate immunity in rat kidneys. Transplantation 2005;79:1370-7.
126. Shigeoka AA, Holscher TD, King AJ, Hall FW, Kiosses WB, Tobias PS, Mackman N, McKay DB. TLR2 is constitutively expressed within the kidney and participates in ischemic renal injury through both MyD88-dependent and -independent pathways. J Immunol 2007;178:6252-8.
127. Chakraborti T, Das S, Mondal M, Roychoudhury S, Chakraborti S. Oxidant, mitochondria and calcium: an overview. Cell Signal 1999;11:77-85.
128. Nakano H, Nakajima A, Sakon-Komazawa S, Piao JH, Xue X, Okumura K. Reactive oxygen species mediate

crosstalk between NF-kappaB and JNK. Cell Death Differ 2006;13:730-7.
129. Matsuzawa A, IchijoH. Stress-responsive protein kinases in redox-regulated apoptosis signaling. Antioxid Redox Signal 2005;7:472-81.
130. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 1997;275:90-4.
131. Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, Minowa O, Miyazono K, Noda T, Ichijo H. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2001;2:222-8.
132. Wang X, McCullough KD, Franke TF, Holbrook NJ. Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. J Biol Chem 2000;275:14624-31.
133. Stefanelli C, Tantini B, Fattori M, Stanic' I, Pignatti C, Clo C, Guarnieri C, Caldarera CM, Mackintosh CA, Pegg AE, Flamigni F. Caspase activation in etoposide-treated fibroblasts is correlated to ERK phosphorylation and both events are blocked by polyamine depletion. FEBS Lett 2002;527:223-8.
134. Sarró E, Tornavaca O, Plana M, Meseguer A, Itarte E. Phosphoinositide 3-kinase inhibitors protect mouse kidney cells from cyclosporine-induced cell death. Kidney Int 2008;73:77-85.
135. Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. J Biol Chem 1996;271:4138-42.
136. Hung CC, Ichimura T, Stevens JL, Bonventre JV. Protection of renal epithelial cells against oxidative injury by endoplasmic reticulum stress preconditioning is mediated by ERK1/2 activation. J Biol Chem 2003;278:29317-26.
137. Hu P, Han Z, Couvillon AD, Exton JH. Critical role of endogenous Akt/IAPs and MEK1/ERK pathways in counteracting endoplasmic reticulum stress-induced cell death. J Biol Chem 2004;279:49420-9.
138. Mkaddem SB, Werts C, Goujon JM, Bens M, Pedruzzi E, Ogier-Denis E, Vandewalle A. Heat shock protein gp96 interacts with protein phosphatase 5 and controls toll-like receptor 2 (TLR2)-mediated activation of extracellular signal-regulated kinase (ERK) 1/2 in post-hypoxic kidney cells. J Biol Chem 2009;284:12541-9.
139. von Kriegsheim A, Pitt A, Grindlay GJ, Kolch W, Dhillon AS. Regulation of the Raf-MEK-ERK pathway by protein phosphatase 5. Nat Cell Biol 2006;8:1011-6.
140. Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol 2001;3:891-6.
141. Yang Y, Liu B, Dai J, Srivastava PK, Zammit DJ, Lefrançois L, Li Z. Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. Immunity 2007;26:215-26.
142. Cunningham PN, Wang Y, Guo R, He G, Quigg RJ. Role of Toll-like receptor 4 in endotoxin-induced acute renal failure. J Immunol 2004;172:2629-35.
143. Ducloux D, Deschamps M, Yannaraki M, Ferrand C, Bamoulid J, Saas P, Kazory A, Chalopin JM, Tiberghien P. Relevance of Toll-like receptor-4 polymorphisms in renal transplantation. Kidney Int 2005;67:2454-61.
144. Palmer SM, Burch LH, Mir S, Smith SR, Kuo PC, Herczyk WF, Reinsmoen NL, Schwartz DA. Donor polymorphisms in Toll-like receptor-4 influence the development of rejection after renal transplantation. Clin Transplant 2006;20:30-6.
145. Nogueira E, Ozaki KS, Macusso GD, Quarim RF, Câmara NO, Pacheco-Silva A. Incidence of donor and recipient toll-like receptor-4 polymorphisms in kidney transplantation. Transplant Proc 2007;39:412-4.
146. Braudeau C, Ashton-Chess J, Giral M, Dugast E, Louis S, Pallier A, Braud C, Moreau A, Renaudin K, Soulillou JP, Brouard S. Contrasted blood and intragraft toll-like receptor 4 mRNA profiles in operational tolerance versus chronic rejection in kidney transplant recipients. Transplantation 2008;86:130-6.
147. Krüger B, Krick S, Dhillon N, Lerner SM, Ames S, Bromberg JS, Lin M, Walsh L, Vella J, Fischereder M, Krämer BK, Colvin RB, Heeger PS, Murphy BT, Schröppel B. Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. Proc Natl Acad Sci USA 2009;106:3390-5.
148. Lane MC, Lockatell V, Monterosso G, Lamphier D, Weinert J, Hebel JR, Johnson DE, Mobley HL. Role of motility in the colonization of uropathogenic *Escherichia coli* in the urinary tract. Infect Immun 2005;73:7644-56.
149. 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 USA 2007;104:16669-74.
150. Wright KJ, Seed PC, Hultgren SJ. Uropathogenic *Escherichia coli* flagella aid in efficient urinary tract colonization. Infect Immun 2005;73:7657-68.
151. Pichon C, Háchard C, du Merle L, Chaudray C, Bonne I, Guadagnini S, Vandewalle A, Le Bouguénec C. Uropathogenic *Escherichia coli* AL511 requires flagellum to enter renal collecting duct cells. Cell Microbiol 2009;11:616-28.
152. Hawn TR, Scholes D, Li SS, Wang H, Yang Y, Roberts PL, Stapleton AE, Janer M, Aderem A, Stamm WE, Zhao LP, Hooton TM. Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women. PLoS One 2009;4:e5990.