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)*

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...
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, and are also the most common bacterial complication in renal transplant patients. 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*, the ability of uropathogenic *E. coli* (UPECs) to form biofilms, the development of intracellular bacterial communities of UPECs, and the long-term consequences of complicated UTI (i.e. acute pyelonephritis, APN) for renal graft function. 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 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. 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), the Nacht-LRR (NLR) receptors, including Nod1 and Nod2, intracellular proteins that sense the presence of invasive bacteria, the RIG-like receptors (RLRs), and mannose-binding lectins. 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.

Epithelial cells also sense the presence of endogenous molecules, known as damage-associated molecular patterns (DAMPs), that are released by stressed cells. 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. 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. TLR/ligand interaction leads to the recruitment and activation of adaptor molecules. We will make no attempt to describe the detailed molecular mechanisms underlying TLR signaling here. 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 TAK1 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. 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). 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. 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. Tsuboi et al. first reported the expression of TLRs 1, 2, 4, and 6 in primary cultures of mouse cortical renal epithelial cells. Other studies have confirmed that TLR2 and TLR4 are expressed in renal tubule cells from mouse, rat, and human kidneys. TLR5 has also been shown to be expressed in the mouse bladder and, to a lesser extent, in mouse kidneys. 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, but not in primary cultures of mouse cortical renal epithelial cells. 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. TLR11 has also been shown to recognize the profilin-like protein from Toxoplasma gondii. 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

<table>
<thead>
<tr>
<th>TLR</th>
<th>Tubule cell expression</th>
<th>Exogenous PAMP ligands</th>
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<tr>
<td>1</td>
<td>ARN</td>
<td>Triacyl lipopeptides</td>
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<tr>
<td>2</td>
<td>ARN, protein</td>
<td>Lipoproteins/lipopeptides</td>
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<td></td>
<td></td>
<td>Lipoteichoic acid</td>
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<td></td>
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<td>Glycolipids</td>
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<td></td>
<td></td>
<td>Porins</td>
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<td></td>
<td></td>
<td>Atypical LPS (Leptospira)</td>
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<td></td>
<td></td>
<td>Zymosan</td>
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<tr>
<td>3</td>
<td>ARN</td>
<td>Double stranded RNA (virus)</td>
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<tr>
<td></td>
<td></td>
<td>LPS</td>
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<td></td>
<td></td>
<td>Taxol</td>
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<tr>
<td>4</td>
<td>ARN, protein</td>
<td>Retroviral envelop proteins</td>
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<td></td>
<td></td>
<td>Microbial Hsp</td>
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<tr>
<td>5</td>
<td>ARN</td>
<td>Flagellin</td>
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<tr>
<td>6</td>
<td>ARN</td>
<td>Diacyl lipopeptides</td>
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<td></td>
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<td>Zymosan</td>
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<td></td>
<td></td>
<td>Double-stranded RNA</td>
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<tr>
<td>11*</td>
<td>ARN</td>
<td>Profilin-like protein (T. gondii)</td>
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<td></td>
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<td>E. coli</td>
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*: 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. Using reciprocal chimeric mice, Robson’s group showed that in addition to neutrophils, 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-
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. TLR4 mutations linked to LPS hyporesponsiveness have been also identified in humans. 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. Similar findings have been also reported in models of intestinal mucosal inflammation. Several enteroctye 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. Cellular TLR4 has been reported to play a critical role in the pathogenesis of necrotizing enterocolitis. Moreover, activation of TLR4 signaling has been shown to promote the internalization and phagocytosis of E. coli, 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. 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). 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. They also express P-fimbriae, which bind to the glycosphingolipids that are abundant on the surface of renal epithelial cells.

**Epithelial TLR4 and ascending urinary tract infections**

The attachment of *E. coli* to bladder epithelial cells induces a potent TLR4-mediated inflammatory response. 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, which consist mainly of intercalated cells. Analysis of pri-

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**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). 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). 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). 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, particularly in patients exhibiting elevated levels of circulating AVP.

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

Although the process of UPEC invasion of bladder epithelial cells has been extensively studied, 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, which is highly expressed in the caveolae, a sub-domain of the biochemically defined lipid raft. 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. Using non-pathogenic and pathogenic strains, we identified two modes of cellular translocation of UPECs across the renal tubule epithelial barrier. The UPEC strain CFT073, which expresses cytolytic and vacuolating cytolysins, 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.

**The role of epithelial TLR4 in mediating drug-induced nephotoxicity**

Cisplatin is a potent cancer chemotherapy drug that can induce acute renal failure. Cisplatin and LPS induce synergistic, TLR4-mediated, renal toxicity. Cisplatin-treated, wild-type mice exhibit greater renal cell damage than their cisplatin-treated, TLR4 deficient mouse counterparts. Using a bone-marrow chimeric approach, Zhang et al. have shown...
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 \(\alpha\)-defensins HNP1-4 are expressed in neutrophils,\(^{(81)}\) and \(\beta\)-defensins HD5 and HD6 are expressed in Paneth cells located in the crypts of the small intestine.\(^{(82)}\) No there is normally

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**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 \(\alpha\)-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-\(\kappa\)B (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.
no spaces between, HNP1-4. Cathelicidin is widely expressed in leukocytes and epithelial cells. Both defensins and cathelicidin are expressed in the urinary tract and epididymis, 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. 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.

**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. THP, which stimulates pro-inflammatory mediators in human monocytes and granulocytes, activates dendritic cells via a TLR4-dependent mechanism, 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. THP was also shown to protect kidneys against ischemic injury by reducing the inflammatory response, possibly through TLR4-mediated signaling.

**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. Lipocalin-deficient mice were also shown to be more susceptible to systemic E. coli infection, but not to I/R injury. 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. They include the high-mobility group box 1 (HMGB1), heat shock proteins, fibronogen, fibronectin, surfactant, β-defensin, uric acid, hyaluronan, and the matrix components biglycan and heparan sulfate. Most of them have been shown to activate TLR2 and TLR4. Genomic DNA from dying cells also induces the maturation of antigen-presenting cells and self-mRNA released from necrotic cells activates TLR3. HMGB1, which is ubiquitously expressed in eukaryotic cells, is a member of the non-histone, chromatin-associated, high-mobility group (HMG) family of proteins. HMGB1, one of the DAMPs that has undergone the most extensive investigation, is released by all cells in a context of necrotic cell death. It can also be secreted by macrophages or dendritic cells in response to LPS, interferon-γ, and TNF-α. Like TLR2, TLR4 recognizes a wide range of DAMPs (Table 2). TLR4 has

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<tr>
<th>Endogenous DAMP Ligands Activating TLR Signaling</th>
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<tr>
<td>Heat shock proteins</td>
</tr>
<tr>
<td>(Hsp60, Hsp70, Hsp90, gp96)</td>
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<tr>
<td>Fibronectin</td>
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<tr>
<td>Hyaluronic acid</td>
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<tr>
<td>Heparan sulfate</td>
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<tr>
<td>Fibrinogen</td>
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<td>Surfactant Protein-A</td>
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<td>β-defensins</td>
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<td>High mobility group box 1 (HMGB1)</td>
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<td>Uric acid</td>
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been implicated in the mucosal inflammation caused by ischemia/reperfusion (I/R) in hepatic I/R, cardiac I/R, and hemorrhagic shock. TLR4 and TLR2 also mediate inflammatory responses during I/R injury. The expression levels of both TLR2 and TLR4 increase rapidly in post-ischemic renal tubule epithelial cells (RTECs). Conversely, TLR2- and TLR4-deficient mice are better protected than wild-type mice. 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. 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. 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, 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. 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. 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. The activation of ERK has been shown to be associated with drug-induced apoptosis, but has also been implicated in cell survival following oxidant injury or the induction of endoplasmic reticulum (ER), stress-induced, cell death signaling.

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. 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. 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, 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, 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. 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. 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. Recently, Krüger et al. have shown that the TLR4 ligand HMGB1, 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
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. 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, 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, 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. Taken together these studies provide evidence that TLR5 is involved in controlling 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.\textsuperscript{(152)}

Identification of the proteins involved in controlling 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.

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The paper discusses the role of heat shock protein gp96 in innate immunity and its effects on cell viability. gp96 is required for innate immunity but not cell viability. The paper also highlights the role of gp96 in the stress-responsive protein kinases and its importance in the innate function of macrophages. Additionally, the paper mentions the regulation of the Raf-MEK-ERK pathway by protein phosphatase 5 and its role in protecting renal epithelial cells against oxidative injury by endoplasmic reticulum stress preconditioning.