Signaling Danger: Toll-Like Receptors and their Potential Roles in Kidney Disease

HANS-JOACHIM ANDERS,* BERNHARD BANAS,† and DETLEF SCHLÖNDORFF*
*Nephrological Center, Medizinische Poliklinik, Klinikum der Universität München-Jenhausen, Germany; and †Klinik u. Poliklinik f. Innere Medizin II, Universität Regensburg, Germany.

Abstract. Toll-like receptors (TLR) are an emerging family of receptors that recognize pathogen-associated molecular patterns and promote the activation of leukocytes and intrinsic renal cells. Ligands of the TLR include exogenous microbial components such as LPS (TLR4), lipoproteins and peptidoglycans (TLR1, -2, -6), viral RNA (TLR3), bacterial and viral unmethylated cytosin-guanosin dinucleotide (CpG)-DNA (TLR9), and endogenous molecules including heat-shock proteins and extracellular matrix molecules. Upon stimulation, TLR induce expression of inflammatory cytokines or costimulatory molecules via the MyD88-dependent and MyD88-independent signaling pathways shared with the interleukin-1 receptors. TLR are differentially expressed on leukocyte subsets and non-immune cells and appear to regulate important aspects of innate and adaptive immune responses. Tubular epithelial cells are among the non-immune cells that express TLR1, -2, -3, -4, and -6, suggesting that these TLR might contribute to the activation of immune responses in tubulointerstitial injury (e.g., bacterial pyelonephritis, sepsis, and transplant nephropathy). In addition, TLR9 has been shown to be involved in antigen-induced immune complex glomerulonephritis and lupus nephritis by regulating humoral and cellular immune responses. TLR are evolutionary conserved regulators of innate and adaptive immune responses. It is likely that TLR are involved in many if not all types of renal inflammation. Here the authors provide an overview on the biology of TLR, summarize the present data on their expression in the kidney, and provide an outlook for the potential roles of TLR in kidney disease.

Infection is commonly associated with a decline of renal function. Infections can trigger the onset of immune complex glomerulonephritis (GN), transplant rejection, or a worsening of underlying GN and renal vasculitis. As most of these kidney diseases lack direct pathogen invasion, it is thought that the activation of systemic and local immune responses contributes to renal dysfunction, but the mechanisms of this activation are poorly understood.

The recent discovery of the toll-like receptor (TLR) family has focused attention on the disease processes as TLR mediate pathogen recognition and immune activation (1,2). Both immune and non-immune cells express inflammatory cytokines and chemokines upon stimulation of the TLR with their specific ligands. These ligands include LPS or other pathogen-associated molecular patterns (PAMP). Although it is likely that TLR are involved in the pathogenesis of many diseases, a paucity of data exists on their expression in the kidney and their role in renal disease. Stimulated by these new concepts of immune activation and first studies on the role of TLR in kidney disease, we have summarized an outlook on the potential roles of TLR in infection- and injury-associated deterioration of renal function and kidney diseases in general.

Danger Recognition by Pattern Recognition Receptors: An Ancient Concept of Defense

Our general understanding of the immune system is based on the assumption that self and foreign must be distinguished to preserve tolerance to self and to defend against foreign. This model has been questioned because it fails to integrate several observations. Certain “foreign” tissues or organisms in the body do not induce defense reactions as one would expect from this model such as the fetus during pregnancy or most bacteria and fungi in the intestine. For the nephrologist, it is surprising that kidney transplants from MHC-mismatched living donors show a better outcome than kidneys from matched cadavers even though the degree of the “foreigness” is comparable (3). Furthermore, systemic autoimmunity involves activation of humoral and cellular immune responses as in systemic lupus (SLE), although this is not caused by foreign antigens, an unknown process triggers a loss of self-tolerance.

Triggering immune responses relies on the specific recognition of a pathogen or foreign antigen, or as it has been proposed in a more general sense, a danger signal (4–6). In any case, the trigger mechanisms for innate and adaptive immune responses are different (Table 1). Innate immunity involves binding of the pathogen by so-called pattern recognition receptors (PRR) to enable danger signaling. Macrophages and dendritic cells use families of non-clonal PRR, including TLR that are genetically defined in their molecular and peptide
structure and provide the best distinction between the self and the non-self that has been selected by evolution (Table 1 [7–9]). Within the PRR, the TLR family is specifically involved in immune cell activation because they all can activate NFκB-dependent gene expression on the basis of the recognition of their specific ligands (2,10).

Interestingly, TLR also recognize endogenous ligands (11). Endogenous danger signal can be provided by injured cells or tissue fragments representing a second level for monitoring tissue homeostasis in addition to the distinction between self and foreign based on T cells (1,12,13). In this context, apoptotic cell death is important to avoid danger signaling (e.g., by necrotic cell debris during tissue injury) (14). Therefore, the tissues themselves appear to be critical components of tolerance since normal tissue homeostasis, including the appropriate apoptotic cell removal avoids danger signaling and stimulation of the innate immune system (1,12). In contrast, tissue injury provides endogenous and exogenous ligands that activate TLR on immune and non-immune cells (1,15). For example, cadaver kidneys may experience ischemic or septic injury which might provide danger signals to the host’s innate immune system and result in an exaggerated immune response (16). In contrast, the lack of significant ischemia or other injury in living unrelated renal transplants could translate into less danger signaling and avoid this additional immune activation via TLR, a hypothesis that remains to be proven.

### The TLR Family

To date, 10 human and 9 murine proteins related to the Drosophila Toll gene have been characterized (Figure 1 [1,17]). The specificity of TLR signaling within the family of TLR depends on heterodimerization of certain TLR and on a group of cytoplasmatic adaptor molecules (17,18). The first recognized adaptor molecule was myeloid differentiation factor MyD88 (19–22). Studies with MyD88-deficient mice revealed other MyD88-independent signaling pathways involving MyD88-adapter-like (Mal) protein (23,24) or the TIR domain-containing adaptor molecules TRIF (24) and TRAM (18). MyD88 seem to be the only adaptor molecule for Toll9, but TLR1, -2, -4, and -6 can use Mal or MyD88, and TLR3 and TLR4 activation can involve MyD88 or TICAM-1 (23,25,26).

#### Ligand Interactions of TLR

**TLR2, TLR1, and TLR6**

TLR2 is essential for the signaling of a variety of ligands (Table 2 [27–34]). For example, TLR2 responds to peptidoglycan, a main wall component of Gram-positive bacteria (27), lipopeptides, and lipoproteins. TLR2 cooperates with other TLR family members, in particular, TLR1 and TLR6, which contributes to the discrimination among different microbial components. TLR1 interacts with TLR2, and coexpression of TLR1 and TLR2 enhance NFκB activation in response to triacylated lipopeptides (35,36) on human monocytes (33).
TLR1 and TLR2 heterodimerization activates dendritic cells, B cells, NK cells (37), mast cells (38), and keratinocytes (39), but not T cells (40). Macrophages from TLR1-deficient mice showed impaired proinflammatory cytokine production in response to a 19-kD mycobacterial lipoprotein and other synthetic triacylated lipopeptides. In contrast, heterodimerization of TLR2 with TLR6 seems to be required for the detection of yeast zymosan and diacylated mycoplasmal lipopeptides (10,41).

Recently, endogenous TLR2 ligands have been identified (Table 3 [11]). Necrotic but not apoptotic cells activate fibroblasts and macrophages via TLR2 (42). This could be mediated by uptake of intracellular proteins such as heat shock protein (HSP) 70 shown to induce IL-12 expression through TLR2 (43). Therefore the recognition of molecules that are expressed during cellular injury may modulate the function of non-immune cells.

**TLR3**

TLR3 has shown to mediate the response to single- and double-stranded viral RNA (44). TLR3 activation leads to an induction of antiviral and proinflammatory cytokines and dendritic cell maturation. The effects of TLR3 activation are also modulated by the specific RNA sequences, as polyA:polyU RNA tends to induce predominately IgG2a antibody production, whereas polyI:polyC RNA induce predominately IgG1

---

**Table 2.** Toll-like receptors, their expression on human leukocytes, and their exogenous ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>TLR+ Immune Cells</th>
<th>Ligand</th>
<th>Source of the Ligand</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>M, DC, B, MC, NK</td>
<td>Soluble factors</td>
<td>Neisseria meningitides</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triacylated lipoproteins</td>
<td>Mycobacteria</td>
<td>(151)</td>
</tr>
<tr>
<td>TLR2</td>
<td>M, B, NK, DC</td>
<td>Lipoproteins, peptidoglycan, Modulin</td>
<td>Gram-positive bacteria</td>
<td>(152, 153)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycolipids, lipoproteins</td>
<td>Staphylococcus</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LPS</td>
<td>Spirochetes</td>
<td>(154, 155)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoarabinomannan</td>
<td>Spirochetes, H. pylori</td>
<td>(34, 155)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoproteins/peptides</td>
<td>Mycobacteria</td>
<td>(156, 157)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zymosan</td>
<td>Other bacteria</td>
<td>(152)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GPI anchors</td>
<td>Yeast</td>
<td>(10, 158)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer membrane protein A</td>
<td>Trypanosoma cruzi</td>
<td>(31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soluble factors</td>
<td>Klebsiella</td>
<td>(32, 47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannuronic acid polymers</td>
<td>Neisseria meningitides</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dsRNA, ssRNA</td>
<td>Pseudomonas aerug.</td>
<td>(30)</td>
</tr>
<tr>
<td>TLR3</td>
<td>DC</td>
<td>LPS</td>
<td>Viruses</td>
<td>(15, 44)</td>
</tr>
<tr>
<td>TLR4</td>
<td>M, N</td>
<td>Lipoteichoic acids</td>
<td>Gram negative bacteria</td>
<td>(52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taxol</td>
<td>Gram positive bacteria</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F protein</td>
<td>Plants</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannuronic acid polymers</td>
<td>RS Virus</td>
<td>(58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flagellin</td>
<td>Pseudomonas aerug.</td>
<td>(30)</td>
</tr>
<tr>
<td>TLR5</td>
<td>M, DC</td>
<td>Modulin</td>
<td>Bacteria with flagella</td>
<td>(65, 67)</td>
</tr>
<tr>
<td>TLR6</td>
<td>M</td>
<td>Zymosan</td>
<td>Staphylococcus</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer surface protein A</td>
<td>Yeast</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diacetylated lipopeptides</td>
<td>Borrelia burgdorferi</td>
<td>(154)</td>
</tr>
<tr>
<td>TLR7</td>
<td>M, DC</td>
<td>Imidazoquinolines</td>
<td>Mycobacteria</td>
<td>(36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guanosin analogs</td>
<td>Antiviral compounds</td>
<td>(70, 71)</td>
</tr>
<tr>
<td>TLR8</td>
<td>TLR8</td>
<td>Imidazoquinolines</td>
<td>Antiviral compounds</td>
<td>(70)</td>
</tr>
<tr>
<td>TLR9</td>
<td>DC, B</td>
<td>Unmethylated CpG DNA</td>
<td>Bacteria, viruses</td>
<td>(73)</td>
</tr>
<tr>
<td>TLR10</td>
<td>B</td>
<td>?</td>
<td>?</td>
<td>(78)</td>
</tr>
</tbody>
</table>

* M, monocytes; NK, NK cells; B, B cells; T, T cells; MC, mast cells; DC, dendritic cells; N, neutrophils.

---

**Table 3.** Toll-like receptors and their endogenous ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>Ligand</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Oxygen radicals</td>
<td>(126)</td>
</tr>
<tr>
<td></td>
<td>Necrotic cells</td>
<td>(42)</td>
</tr>
<tr>
<td>TLR4</td>
<td>HSP60 (?), HSP70; GP96</td>
<td>(43, 59, 60, 130)</td>
</tr>
<tr>
<td></td>
<td>Fibronectin EDA domain</td>
<td>(62)</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td>Heparan sulfate</td>
<td>(61)</td>
</tr>
<tr>
<td></td>
<td>Hyaluronan</td>
<td>(64)</td>
</tr>
<tr>
<td></td>
<td>Lung surfactant protein A</td>
<td>(159)</td>
</tr>
<tr>
<td></td>
<td>β-defensin 2</td>
<td>(51)</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG chromatin-IgG complexes</td>
<td>(75)</td>
</tr>
</tbody>
</table>
antibodies (15). It appears that viral RNA can act as a natural adjuvant that may promote the loss of tolerance against endogenous or exogenous antigens and modulates the Th1/Th2 balance of the specific immune response (15). There seem to be considerable differences in TLR3 expression on leukocyte subsets between humans and rodents as TLR3 is expressed on murine macrophages; whereas, in humans TLR3 is exclusively expressed on myeloid dendritic cells (44–46). Recently TLR3 has been shown to be expressed on mouse and human kidney tissues (47,48). One source of renal TLR3 expression could be tubular epithelial cells. These cells appear to express TLR3 constitutively at low levels and upregulate TLR3 upon challenge with LPS in vitro (49).

**TLR4**

TLR4 is considered to be the critical component of the LPS receptor complex. But this receptor can also bind a variety of other ligands (50). In vivo LPS can cause endotoxic shock by inducing the release of proinflammatory cytokines and chemokines from immune and non-immune cells. This may be exclusively mediated by TLR4. β-defensin 2 is produced in response to microbial infection of mucosal tissue or skin and activates dendritic cells via TLR4 (51). In 1998, a point mutation in the TLR4 gene was found to be the molecular basis of LPS hyporesponsiveness in C3H/HeJ mice (52). The same phenotype is seen in C57BL/10ScCr mice, which have a chromosomal defect that leads to a loss of the whole TLR4 gene (53). These mice show a similar phenotype as mice with a targeted disruption of the TLR4 gene (54). In humans, TLR4 mutations also are associated with impaired responsiveness to LPS (55), but lack of TLR4 in humans does not affect the outcome of bacterial sepsis (56). TLR4 recognizes not only LPS but also PAMP distantly related to LPS (Table 2, [57,58]). It was recently suggested that endogenous ligands may act through TLR4 (Table 3 [11]). HSP60 and HSP70 have shown to activate NFκB mobilization and mitogen-activated protein kinases (MAPK) pathways through their direct binding to TLR4 (43,59,60). Extracellular matrix breakdown products such as hyaluronan, heparan sulfate, fibrinogen, or the fibronectin EDA domain can also activate TLR4 (61–64). However, whether these data relate to LPS contamination of ligand preparation remains controversial (65). Nevertheless, it is hypothesized that TLR4 recognizes endogenous molecules that are exposed during cellular injury and extracellular matrix remodeling. This suggests that TLR4 signaling may also be involved in signaling during tissue injury that is independent of pathogen invasion.

**TLR5**

TLR5 has been shown to bind bacterial flagellin from both Gram-positive and Gram-negative bacteria (66). To date, this is the only ligand for this receptor that has been identified. TLR5 induces maturation of human but not murine dendritic cells (67). In addition to immune cells, TLR5 is also expressed on the basolateral but not on the apical membrane of human intestinal epithelial cells. This could explain why pathogenic *Salmonella*, that translocate flagellin across epithelia, but not commensally *Escherichia coli*, can activate epithelial proinflammatory gene expression (68). In contrast, murine renal tubular epithelial cells seem not to express TLR5 in culture (49).

**TLR7 and TLR8**

In contrast to other TLR, TLR7, -8, and -9 are thought to act intracellularly in endosomes and bind to their ligands after phagocytosis and lysis of a surrounding envelope (50,69). TLR7 and TLR8 are highly homologous to TLR9, although their natural ligands are still unclear (17). Several guanosine analogs induce proinflammatory cytokines and activate dendritic cells via TLR7 (70). As TLR7-deficient mice do not respond to imidazoquinoline compounds, synthetic antiviral compounds with nucleic acid–like structure, it has been proposed that TLR7, similar to TLR9, participates in the discrimination of nucleic acid-like structures (71).

**TLR9**

TLR9 binds unmethylated cytosin-guanosin dinucleotide (CpG)-DNA, a dinucleotide sequence that has been identified as a stimulatory motif of bacterial and viral DNA (72). CpG motifs are common in DNA of bacteria and viruses, but they are underrepresented in vertebrate DNA, which is usually methylated. CpG rich DNA and oligodeoxynucleotides carrying the CpG motif induce immunostimulatory activities on B cells and antigen-presenting cells (APC) exclusively via TLR9, as TLR9-deficient mice lack these responses (73). TLR9 signaling on B cells or dendritic cells may be enhanced by CpG-DNA–conjugated proteins such as IgG or ovalbumin, thereby enhancing an antigen-specific humoral or cellular immune response of the Th1 type (74,75).

**TLR10**

TLR10 is expressed on human B cells after activation of the B cell receptor. However, ligands for human TLR10 as well as the effects of TLR10 activation remain to be identified (76,77). TLR10 seems not to be expressed in the healthy kidney (48,78).

**Biologic Effects Induced by TLR Activation on Immune Cells**

**Activation of APC**

APC that express various TLR, including TLR1, -2, -4, and -6, include murine dendritic cells and microglia (79–81). TLR activation may therefore play a major role during septic shock, systemic inflammatory response syndromes, and local immune responses (8,82–90). In macrophages, the expression of CC-chemokines and CC-chemokine receptors by TLR9 activation may contribute to additional leukocyte infiltration to the site of injury as it has been observed in several disease models, including GN (87–90).

TLR are also involved in dendritic cell maturation induced by exogenous pathogens. Interestingly, dendritic cell subpopulations show different TLR expression profiles (91,92). Human plasmacytoid dendritic cells express TLR1, -6, -7, and -9, the
latter being most prominent (37). In contrast, myeloid dendritic cells express TLR4, -7, and -3 but not TLR9 (46). Stimulation of TLR4 or TLR9 on dendritic cells induces IL-12 and IFN-γ secretion as well as surface expression of costimulatory molecules, including CD40, CD80, and CD86 (64,79,81,93–95). TLR activation with their specific TLR ligands induces dendritic cell maturation, CCR7 expression (95) and cytokine secretion increase antigen-presenting capacity and migration from peripheral tissues to lymphnodes, where the dendritic cells interact with and specifically stimulate T cells (95,96).

Thus, activation of TLR such as TLR4 and TLR9 induce maturation of both dendritic cells and tissue macrophages, but this process leads to different functional activation that relates to the specific function of these cells (Figure 2). Macrophage stimulation supports local cytokine and chemokine secretion that contributes to local inflammation and leukocyte accumulation. In contrast, maturation of dendritic cells leads to emigration from the site of injury to the regional lymphnodes to convey antigens and proinflammatory signals to T cells, which is a prerequisite for the adaptive immune response.

**Activation of B Cells**

Human B cells express high amounts of TLR7, -9, and -10, but they lack or show variable expression of the other TLR (37,48,71). In 1995, Krieg et al. (72) described the stimulatory effects of CpG-DNA on B cells, which was later found to be exclusively mediated via TLR9 (73). TLR9 activation induces proliferation of B cells, which is associated with the inhibition of B cell receptor–induced apoptosis (97–99). Furthermore, TLR9 stimulates NFkB-dependent expression of IL-6, IL-10, IL-12 and costimulatory molecules such as MHC class II, CD80, and CD86 (Figure 3 [72,100,101]). Crosslinking of the surface B cell receptors with TLR9 by immunocomplexes that contain chromatin triggers T cell-independent proliferation of B cells and secretion of IgG and IgM autoantibodies (75,102).

This mechanism may play an important role in systemic autoimmunity (103). B cells require two different signals to regulate the isotype of secreted IgG (104). In the presence of the Th2 cytokine, IL-4 CpG-DNA promotes IgG1 and IgE secretion, whereas in the presence of the Th1 cytokine IFN-γ B cells secrete IgG2a (100). However, in vivo the activation of TLR9 appears to induce a Th1-like response due to the predominant secretion of IFN-γ, IL12, and other Th1 cytokines by macrophages and dendritic cells (Figure 3 [105]).

**Polarization of Adaptive Immune Responses by TLR Activation**

TLR stimulation has been shown to induce different cytokine patterns that are associated with either predominant Th1 or Th2-like immune responses (93). This may also depend on the mouse strain studied (92). While in isolated dendritic cells from mouse spleens, TLR2 activation favors a predominant Th2-like response and TLR4 activation a Th1-like response, considerable overlap exists (106). For example, TLR4 appears to be required for Th2 responses, suggesting a complex interplay of stimulatory signals (107). In vivo, the overall immune response...
response will be further modulated by a large number of molecules. To date, in vivo studies are only available for CpG-DNA–TLR9 interaction (97), which appears to be most relevant for costimulatory cytokine T cell responses (108). TLR9 activation is followed by a robust Th1 humoral and cellular immune response in vivo (105,108–112), which identified synthetic oligodeoxynucleotides containing stimulatory CpG motifs as a potential tool for the development of novel immunomodulatory treatment strategies (113,114). These include CpG-DNA as vaccine adjuvants (108,115,116), as immune modulators of atopy (117), as inducers of cell responses against malignancy (109,118), and as modulators of the immune response during infection with intracellular microbes (119).

Implications of TLR Activation for the Pathogenesis of Kidney Disease

The basic mechanisms of danger recognition via TLR signaling in response to infectious and non-infectious cellular damage may also be involved in renal disease. To date, however, only limited studies have addressed this issue. Nevertheless, initial data point toward a contribution of TLR to the pathogenesis of a number of renal diseases (Table 4).

Sepsis and Renal Infection

Mouse renal tubular cells in culture constitutively express TLR1, -2, -3, -4, and -6 in vitro (49). Upon stimulation with LPS, they upregulate TLR2, -3, and -4 and secrete CC-chemokines such as CCL2/MCP-1 and CCL5/RANTES (49). These data suggest that tubular TLR expression might be involved in mediating interstitial leukocyte infiltration and tubular injury during bacterial sepsis (Figure 3). Furthermore, TLR-dependent activation of circulating immune cells could lead to cytokine production that either directly or indirectly contribute to renal injury. The latter hypothesis is supported by a study with LPS-induced acute renal failure in TLR4-deficient mice. These mice were completely resistant to endotoxin-induced acute renal failure, which was associated with a lack of a systemic TNF-α response (120). Cross transplantation studies showed that TLR4-deficient recipients of wild-type kidneys developed minimal LPS-induced acute renal failure, whereas wild-type recipients of a TLR4-deficient kidney had severe acute renal injury after exposure to endotoxin (120). These data strongly indicate a role for TLR4 activation in acute renal failure of sepsis. However, they do not support the hypothesis that renal TLR4 expression (e.g., on tubular epithelial cells) plays a major role in sepsis-induced acute renal failure. In contrast, they rather implicate a systemic response to sepsis, involving TLR4 and TNFα in the development of acute renal failure during sepsis.

Direct activation of renal cells via TLR, however, occurs by recognition of invading pathogens during renal infection (e.g., during bacterial pyelonephritis). Tubular epithelial cells as well as resident interstitial macrophages or dendritic cells get activated by binding bacterial wall components via TLR as studies with TLR4-deficient mice show decreased interstitial neutrophil infiltration in a model of bacterial pyelonephritis (121). Furthermore, bacterial infections of the kidney induce the secretion of defensins, small antimicrobial peptides, in tubular epithelial cells (122–123). Interestingly, β-defensin 2 has been shown to be an endogenous ligand for TLR4 and to induce dendritic cell activation (51). Ingested bacteria also release CpG-DNA in endosomes, which activate phagocytes via TLR9 in the kidney. Whether these mechanisms also play a role in the pathogenesis of renal diseases that are associated with viral infection such as HIV, CMV, HCV, EBV, or BK virus is unknown to date. However, viral surface proteins as well as viral RNA and DNA bind to TLR in vitro and may therefore also be involved in virus-related kidney disease.

Acute Renal Failure

Acute renal failure (ARF) is caused by ischemic (50%) or nephrotic (35%) injury to the kidney. However, in 50% of the cases of hospital-acquired ARF, the cause is multifactorial or associated with sepsis (124). Nevertheless, irrespective of the initial triggers that lead to tubular cell injury, the activation mechanisms that induce the inflammatory response in the kidney may share a common pathway (124,125). Necrotic tubular cells release potential TLR ligands such as HSP, which could activate other tubular cells or resident immune cells in the kidney (Figure 3). Activation of TLR2 and TLR4 on tubular epithelial cells has been shown to specifically stimulate the NFκB pathway in response to oxidative stress (49,126,127). Furthermore, TLR2 and TLR4 activation on tubular epithelial cells leads to secretion of CC-chemokines (49), indicating a role for these TLR in the initiation of phagocyte influx and immune activation during acute tubular necrosis. Thus TLR activation may be a link between mechanical, toxic, or ischemic tubular cell injury and the onset of an inflammatory “innate” immune response in the pathogenesis of ARF.

Interstitial Fibrosis

During progressive renal fibrosis, increased matrix turnover exposes endogenous TLR ligands such as fibrinogen, heparan sulfate, hyaluron, and fibronectin EDA domain to TLR4 on macrophages (Figure 3 [61–64,128,129]). Other endogenous ligands for TLR2 and TLR4 that may be exposed by necrotic tubular cells during chronic tubulointerstitial injury include HSP and GP96 (59,60,130). Immune cells and intrinsic renal cells that respond to TLR activation could get activated by these extracellular matrix molecules promoting the secretion of inflammatory cytokines and chemokines. This in turn will be followed by additional leukocyte recruitment to the kidney supporting sustained interstitial inflammation and interstitial fibrosis.

Renal Involvement in Systemic Immune Disorders

TLR-mediated immune activation may well play a role in immune complex diseases of the kidney triggered by infections (Table 4). Preliminary data suggest that TLR9 activation by exogenous CpG-DNA does affect the course of immune complex disease. These observations come from recent studies
### Table 4. Possible roles of TLRs in renal disease

<table>
<thead>
<tr>
<th>TLR</th>
<th>Known Ligands</th>
<th>Renal Disease</th>
<th>Possible Ligands</th>
<th>Pathophysiological Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Pathogen wall components, <em>(e.g., lipoproteins)</em> components of necrotic cells</td>
<td>Sepsis-induced ATN, renal infection, CAPD peritonitis</td>
<td>Lipoproteins, peptidoglycans, lipoteichoic acids, zymosan, and other pathogen wall components</td>
<td>Local activation of tubular cells and APC leading to chemokine expression and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemia-reperfusion ATN</td>
<td>Yet undefined necrotic cell components</td>
<td>Local activation of tubular cells and APC leading to chemokine expression and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toxic or immune injury</td>
<td>Yet undefined necrotic cell components</td>
<td>Local activation of tubular cells, and APC leading to chemokine expression and leukocyte infiltration</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA, ssRNA</td>
<td>Hepatitis-associated glomerulonephritis</td>
<td>HCV RNA, other viral RNA</td>
<td>Intrarenal macrophage and dendritic cell activation leading to renal cytokine and chemokine expression</td>
</tr>
<tr>
<td></td>
<td>ssRNA</td>
<td>HIV nephropathy</td>
<td>HIV RNA</td>
<td>Immune cell activation and local injury</td>
</tr>
<tr>
<td>TLR2/4</td>
<td>Pathogen wall components <em>(e.g., LPS), lipoteichoic acids</em></td>
<td>Immune complex GN or renal vasculitis</td>
<td>Infection-triggered disease flares by release of LPS or other pathogen wall components into the circulation</td>
<td>Stimulation of the underlying local immune response, local macrophage activation, cytokine and chemokine expression, and leukocyte infiltration</td>
</tr>
<tr>
<td>TLR4</td>
<td>Pathogen wall components <em>(e.g., LPS), lipoteichoic acids</em></td>
<td>Sepsis-induced tubular necrosis</td>
<td>LPS, lipoteichoic acids, and other pathogen wall components</td>
<td>Local activation of tubular cells and APC leading to chemokine expression and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td>β-defensin 2</td>
<td>Renal infection CAPD peritonitis</td>
<td>Bacterial wall components, β-defensin 2</td>
<td>Local activation of tubular cells and APC leading to chemokine expression and leukocyte infiltration</td>
</tr>
<tr>
<td>TLR9</td>
<td>HSP, fibronectin, fibrinogen, hyaluran heparan sulfate</td>
<td>Interstitial fibrosis, ischemic, toxic, or obstruction-related tubular injury</td>
<td>HSP60, HSP70, GP96 breakdown products of extracellular matrix molecules</td>
<td>Stimulation of specific antibodies, predominant IgG2a, antigen-specific Th1 T cells, local APC activation, chemokine expression, and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td>Unmethylated CpG-DNA</td>
<td>Immune complex GN or renal vasculitis</td>
<td>Pathogen-derived DNA</td>
<td>Stimulation of DNA autoantibody secretion (IgG2a isotype, DNA-specific Th1 T cells), local APC activation, chemokine expression, and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lupus nephritis</td>
<td>Pathogen-derived DNA, Hypomethylated self DNA (UV light-related reduction of DNA methyltransferase enzyme activity)</td>
<td>Stimulation of DNA autoantibody secretion (IgG2a isotype, DNA-specific Th1 T cells), local APC activation, chemokine expression, and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug-induced SLE</td>
<td>Hypomethylated self DNA (drug-related reduction of DNA methyltransferase enzyme activity)</td>
<td>Stimulation of DNA autoantibody secretion (IgG2a isotype, DNA-specific Th1 T cells), local APC activation, chemokine expression, and leukocyte infiltration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HBV-associated vasculitis, GN or lymphoma</td>
<td>HBV DNA</td>
<td>Stimulation of B cell proliferation and HBV antibody secretion (IgG2a, isotype, EBV-specific Th1 T cells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BK virus-induced allograft dysfunction</td>
<td>BK virus DNA</td>
<td>Local APC activation and chemokine expression</td>
</tr>
<tr>
<td>TLR9</td>
<td>Unmethylated CpG-DNA</td>
<td>CMV-induced allograft dysfunction</td>
<td>CMV DNA</td>
<td>Stimulation of BK antibody secretion (IgG2a isotype, BKV-specific Th1 T cells), local APC activation, chemokine expression → transplant rejection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All TLR</td>
<td>Multiple pathogen wall components, including LPS, pathogen-derived DNA/RNA</td>
<td>Renal infection</td>
</tr>
</tbody>
</table>
from our laboratory. We used horse apoferritin (HAF)-induced GN as a model of immune complex GN that is characterized by circulating HAF-specific antibodies, mesangiotrophic GN, glomerular macrophage accumulation, and proteinuria (131). At 14 d, when a marked macrophage infiltrate and proteinuria is present, the early expression of the CC-chemokines CCL2/MCP-1 and CCL5/RANTES chemokine signal is already downregulated (131). The latter was not the case when single doses of 40 μg of CpG-DNA were administered intraperitoneally on days 7 and 8. The sustained glomerular chemokine expression was associated with a marked increase of the number of glomerular macrophages, the extent of glomerular damage, and proteinuria (88). Exogenous CpG-DNA accumulated in glomerular macrophages in mice with GN and TLR9 expression was exclusively found in infiltrating macrophages in nephritic but not mesangial cells or glomeruli of healthy mice. Furthermore, analysis of serum anti-HAF antibody isotypes, mesangial immune deposits, and splenocyte IFN-γ secretion indicated that CpG-DNA induced a Th1 response in mice with HAF GN. Thus bacterial DNA or antigenic wall components such as lipoteichoic acid, endotoxin, or peptidoglycans may aggravate underlying local or systemic immune reactions. This could also involve activation of tissue macrophages that are present irrespective of the defense to a specific pathogen. For example, systemic immune activation could reactivate local, intrarenal “resting” inflammatory responses and cause flares (e.g., in IgA nephropathy, renal vasculitis, or lupus nephritis) (132).

In lupus nephritis, the CpG-DNA receptor TLR9 may have an additional role. It has been suggested that in SLE TLR9 activation by chromatin particles could be involved in directing an adaptive immune response against dsDNA (103,133,134). In SLE, both potential sources of circulating CpG-DNA may be relevant: exogenous microbial DNA and endogenous hypomethylated genomic DNA (72,135). SLE patients have higher serum levels of hypomethylated CpG-DNA that may also relate to reduced methyltransferase activity during disease flares (136,137). Interestingly, known triggers of SLE or lupus-like syndromes such as procainamide, hydralazine, and ultraviolet light are potent inhibitors of DNA methyltransferase activity (138). Thus hypomethylated CpG-DNA may provide an endogenous stimulus for activation of innate and adaptive immune responses that bypass tolerance to self in systemic autoimmune diseases (103). Recent data have shown that coactivation of TLR9 and the IgG receptors on B cells has been described with immune complexes from lupus mice containing also chromatin particles (75). Most interestingly the coactivation of both receptors by the IgG-chromatin immune complexes provides a T-cell–independent signal for proliferation of autoreactive B cells and secretion of autoantibodies (75). Furthermore, DNA autoantibodies induced by bacterial DNA crossreact with methylated vertebrate DNA, and thereby aggravate the humoral disease activity in SLE (139). A role of bacterial CpG-DNA for the pathogenesis of SLE is supported by in vivo studies with CpG-DNA and E. coli DNA in a murine model of lupus nephritis. In MRL lpr/lpr mice, both E. coli DNA and synthetic CpG-DNA induced a marked rise of serum DNA autoantibody titers, predominantly of the IgG2a type (139a). However, stimulation of DNA autoantibody secretion by exogenous DNA does not necessarily lead to aggravation of lupus nephritis, as E. coli DNA improved proteinuria, renal damage, and survival in autoimmune NZB/NZW mice (140). In contrast, in lupus nephritis of MRL lpr/lpr mice, injected E. coli DNA and synthetic CpG-DNA had different effects. In MRL lpr/lpr mice, injected DNA bound to macrophages and CD11c+ dendritic cells in glomeruli and tubulointerstitial areas in nephritic kidneys, which markedly induced CCL2/MCP-1 and CCL5/RANTES expression in those areas (unpublished observation). This was associated with an increase of macrophage and T cell infiltrates compared with control DNA or saline-injected mice. CpG-DNA and E. coli DNA severely aggravated glomerular and tubulointerstitial damage in MRL lpr/lpr mice, which was associated with nephrotic-range proteinuria compared with respective controls. These data demonstrate that in experimental SLE of MRL lpr/lpr mice bacterial CpG-DNA can activate the adaptive immune response against self DNA. Furthermore, circulating bacterial CpG-DNA may then bind to TLR9 on macrophages and dendritic cells in the nephritic kidney, leading to aggravation of renal inflammation, proteinuria, and tissue damage. Whether the discrepancy of the data obtained in MRL lpr/lpr mice and NZB/NZW mice to different pathomechanisms of the SLE in different mouse models remains to be determined. However, in human SLE the release of unmethylated CpG motifs during bacterial infection may act as a costimulus for adaptive immunity against dsDNA but may also directly activate immune cells in the nephritic kidney and thereby aggravate renal inflammation (93,141,142).

In the case of infection with RNA viruses (e.g., hepatitis C virus during cryoglobulinemic GN) activation of TLR3 by the specific recognition of viral ssRNA and dsRNA might mediate similar mechanisms, but experimental evidence for this hypothesis is not available at present (15,44,143–145).

Renal Transplant Rejection

Activation of innate immune responses has long been suspected to be involved in renal transplant rejection (146–148). For example, ischemia-reperfusion injury is an important trigger of acute rejection, and mitigating free radical-mediated reperfusion injury reduced the incidence of rejection episodes (149). It may turn out that endogenous TLR ligands, such as HSP derived from necrotic cells signal danger and act as endogenous immune adjuvants and trigger acute rejection of the allograft. The latter hypothesis is supported by a recent study with MyD88-deficient mice that were unable to reject minor antigen-mismatched (HY-mismatched) skin allografts compared with wild-type controls (150). This was associated with a reduced number of mature dendritic cells in draining lymph nodes, leading to impaired generation of anti-graft reactive T cells and impaired Th1 immunity. These data argue for a role of TLR activation in the transplant setting just like during infections.

Similar mechanisms might be involved in rejection episodes triggered by systemic infections or by infectious organisms that
reside in the kidney, such as cytomegalovirus or polymavirus. If these speculations turn out to be relevant during renal transplant rejection, one may feel that blocking inappropriate immune activation with specific TLR antagonists could represent a new approach for the treatment or prevention of renal allograft rejection.

Summary
Among the PPR, the TLR activate multiple effectors of innate and adaptive immunity upon stimulation with endogenous and exogenous ligands that relate to microbial pathogens or cellular injury. Initial studies suggest that TLR2 and TLR4 can induce chemokine expression by tubular epithelial cells and that TLR9 is expressed on infiltrating APC during immune injury. TLR-mediated immune activation may occur during any type of renal injury by exposure to an increasing number of exogenous or endogenous molecules.

Note Added in Proof

Acknowledgments
We thank Peter J. Nelson for carefully reading the manuscript. The work was supported by grants from the Deutsche Forschungsgemeinschaft (AN 372/4–1) to HJA and BA 2137/1–1 to BB and DS.

References


85. Ohashi K, Burkart V, Flohe S, Kolb H: Heat shock protein 60 is
86. Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose
87. Johnson GB, Brunn GJ, Kodaira Y, Platt JL: Receptor-mediated
88. Feterowski C, Emmanuilidis K, Miethke T, Gerauer K, Rump M,
89. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M,
90. Hacker H, Mischak H, Miethke T, Liptay S, Schmid R, Spar-
91. Means TK, Hayashi F, Smith KD, Aderem A, Luster AD: The
92. Gao B, Tsan MF: Recombinant human heat shock protein 60
93. Means TK, Hayashi F, Smith KD, Aderem A, Luster AD: The
94. Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson
95. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M,
98. Bourke E, Bosisio D, Golay J, Polentarutti N, Mantovani A: The
99. Krieg AM, Yi AK, Matson S, Waldschmidt TJ, Bishop GA,


