The Role of Toll-Like Receptors in the Pathogenesis of Renal Disease

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Toll-like receptors (TLRs) are an essential component of innate immunity, the first line of defense against invading pathogens. However, in addition to activating antimicrobial effector responses directly, TLRs lead to the induction of signals that control the activation of adaptive responses including autoimmune responses and allorecognition. This ability of TLR to control both innate and adaptive immunity has a broad applicability to the development of novel immunotherapies and antimicrobial strategies. This review discusses the basic biology of TLR and their contribution to renal disease.

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The physiologic function of the immune system is to protect individuals from infectious pathogens. The mechanisms that are responsible for this protection fall into 2 broad categories: innate immunity (also called natural or native immunity), which refers to defense mechanisms that are present even before infection and have evolved to specifically recognize microbes and protect multicellular organisms against infection, and adaptive immunity (also called acquired or specific immunity), which consists of mechanisms that are stimulated by microbes and are capable of also recognizing non-microbial substances called antigens. Innate immunity is the first line of defense because it always is ready to prevent and eradicate infections. Adaptive immunity, on the other hand, develops later after exposure to microbes and is even more powerful in combating infections. The major components of innate immunity are epithelial barriers that block entry of environmental microbes, phagocytic cells (eg, neutrophils and macrophages), natural killer cells, and several plasma proteins, including the proteins of the complement system.

Phagocytes recognize microbes by several membrane receptors. These include receptors for mannose residues and N-formyl methionine-containing peptides, which are produced by microbes but not by host cells, and a family of receptors that are homologous to Drosophila proteins called Toll. Different Toll-like receptors (TLRs) are involved in responses to different microbial structures. On recognition of the relevant microbial structure, the TLRs signal by a common pathway that leads to the activation of transcription factors, notably nuclear factor κB (NF-κB), which in turn stimulates production of cytokines and several proteins that are responsible for the microbicidal activities of the phagocytes. Phagocytes that are activated in this way internalize microbes into vesicles, where the microbes are destroyed by reactive oxygen and nitrogen intermediates and hydrolytic enzymes.

Targets of Innate Immune Recognition

The strategy of innate immune recognition is based on the detection of conserved molecular structures produced by microbial pathogens, but not by the host organism. These structures are referred to as pathogen-associated molecular patterns (PAMPs) and represent targets of innate immune recognition. The best-known examples of PAMPs include gram-negative bacterial lipopolysaccharide (LPS), peptidoglycan, bacterial flagellin, unmethylated CpG DNA motifs (found in many bacteria), double-stranded RNA, which is produced by most viruses during the infection cycle, and β-glycans found in fungal cell walls (Table 1). All of these structures are produced by different classes of microbial pathogens, but, importantly, not by the host organisms. Therefore, they function as molecular signatures of microbial metabolism, and their recognition by the innate immune system signals the presence of infection.

Although different PAMPs are not related to each other structurally, they all share several features that reflect the evolutionary strategy of innate immune recognition. First, all
PAMPs are produced by microbes, but not by the host organism. This is the basis of self-/non–self-discrimination that enables innate responses to be mounted only against microbial cells and antigens. Second, PAMPs are invariant among pathogens of a given class. This allows a limited number of Toll receptors to detect any microbial infection. Third, PAMPs often perform physiologic functions that are essential for microbial survival. Therefore, microbial pathogens are limited in their ability to mutate or lose expression of PAMPs to avoid recognition by the immune system.

**TLRs**

The TLRs are membrane proteins that recognize a variety of microbe-derived molecules and stimulate innate immune responses against the microbes. The first protein to be identified in this family was the Drosophila Toll protein, which is involved in establishing the dorsoventral polarity during embryogenesis of the fly. Subsequent studies have shown that Toll also has an essential role in the insect innate immune system. This is the basis of self-/non–self-discrimination that enables innate responses to be mounted only against microbial cells and antigens. Second, PAMPs are invariant among pathogens of a given class. This allows a limited number of Toll receptors to detect any microbial infection. Third, PAMPs often perform physiologic functions that are essential for microbial survival. Therefore, microbial pathogens are limited in their ability to mutate or lose expression of PAMPs to avoid recognition by the immune system.

**TLR Signaling**

Ligand binding to the TLR at the cell surface leads to dimerization of the receptor and its conformational change that is required for the recruitment of downstream signaling molecules, the first of which is the adapter protein MyD88 (Fig. 1). MyD88 is recruited to the cytoplasmic TIR domain where it facilitates the association of a kinase called IL-1 receptor associated kinase 4 (IRAK4). The binding of MyD88 to IRAK4 facilitates phosphorylation of another member of the IRAK family called IRAK1. Activated IRAK1 then undergoes auto-phosphorylation, which enables the signaling molecule, called TNF receptor-associated factor 6 (TRAF-6), to bind to this complex. The IRAK1-TRAF6 complex then dissociates from the receptor and interacts with another preformed complex consisting of transforming-growth-factor-β-activated kinase (TAK) kinase and TAK1-binding protein (TAB1) and TAB2 adaptor proteins. TAK1 subsequently is activated in the cytoplasm, leading to the activation of the I-κB kinase cascade, which in turn leads to activation of the NF-κB transcription factor. The latter induces the transcription of the genes encoding for cytokines, chemokines, and adhesion molecules that are crucial to the inflammatory process and are aimed at clearance of invading bacteria.

Although MyD88 functions as a critical adaptor linking TLRs with downstream molecules, a closer study of MyD88-deficient cells has shown the existence of an MyD88-independent pathway, which also mediates the signaling response to LPS. In particular, TIR-domain-containing adaptor protein inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM) have been
implicated in TLR-mediated activation of the MyD88-independent pathway. In case of TLR4, it is thought that the MyD88-dependent pathway involves the early phase of NF-κB activation whereas the MyD88-independent pathway activates interferon (IFN)-regulatory factor and involves the late phase of NF-κB activation, both of which lead to the production of IFN-α and the expression of IFN-inducible genes.

TLR signaling is regulated negatively by various proteins. The cell-surface receptors ST2 (also known as T1) and single immunoglobulin IL-1R-related molecule (SIGIRR) function as inhibitory receptors, sequestering proteins from signaling complexes and preventing TLR2, TLR4, and TLR9 signaling. IRAK-M, toll-interacting protein (TOLLIP), and a splice variant of MyD88, known as MyD88s, probably interfere with the recruitment and activation of IRAK4 and IRAK1. Recently, TRIAD3A a RING-finger E3 ligase, has been shown to promote ubiquitulation of TLR4 and TLR9, targeting these TLRs for degradation and thereby negatively regulating the intensity and duration of TLR signaling. Therefore, the balance between activation and inhibition is likely to be the key determinant of signal strength.

**Consequences of TLR Activation**

The genes that are expressed in response to TLR signaling encode proteins involved in microbial killing mechanisms. However, in addition to activating antimicrobial effector responses directly, innate immune recognition leads to the induction of signals that control the activation of adaptive immunity including autoimmune responses and allorrecognition (see later). For example, recognition of microbial infection leads to the induction of a local inflammatory response, which is mediated primarily through TLRs expressed in resident macrophages and endothelial cells. Also, recognition of PAMPs by TLRs expressed on dendritic cells induces dendritic cell maturation and an increase in the cell-surface expression of major histocompatibility complex class II and costimulatory molecules (CD80 and CD86). Finally, innate immunity recognition triggers the induction of effector cytokines that control the type of effector responses mounted by the adaptive immune system.

**TLRs and Microbial Infections of the Urinary System**

The urogenital system represents a unique situation in which internal organs potentially are exposed continually to pathogenic microorganisms. TLRs that are expressed on the mucosal surfaces of the urinary tract and in the kidney sense bacteria and provoke an inflammatory response that leads to bacterial clearance. Consistent with this notion, C3HeJ mice that have an inactivating point mutation in the cytoplasmic tail of TLR4, rendering them nonresponsive to LPS, are unable to mount a robust inflammatory response when infected intraurethrally with uropathogenic *Escherichia coli*. Therefore, host-cell recognition of LPS, a primary component of gram-negative bacterial outer membranes, leads to TLR4 activation, which in turn causes stimulation of epithelial production of inflammatory cytokines such as IL-1β, fever-causing IL-6, and neutrophil-recruiting chemokine IL-8. The importance of TLR4 as a primary player in defense against urinary tract infection is underscored further by the recent observation that Tamm-Horsfall protein, the most abundant protein in normal human urine with antibacterial properties, activates professional antigen-presenting cells via a TLR4-dependent mechanism. Therefore, Tamm-Horsfall protein has the ability to activate innate immune responses immediately.

TLR4 expression by the urinary tract epithelium has remained controversial. According to one study, a polymerase chain reaction analysis of total RNA showed that TLR4 was expressed in bladder mucosa but not in human proximal tubule cells. In contrast, immunohistochemical analysis of TLR4 expression in a series of mucosal biopsy specimens obtained from kidneys, ureters, and bladders of patients undergoing urinary tract surgery showed that TLR4 can be detected both in the epithelial cell lining of the urinary tract and in the tubular epithelium of the renal cortex, although the tubular staining was heterogeneous, with stronger staining in some areas. This potentially limited pattern of TLR4 expression may explain the cortical localization of bacteria in pyelonephritis. It should be remembered, however, that other TLR4-positive cell types in the kidney, notably endothelial cells and mononuclear phagocytes, may sense micro-
bial invasion and compensate for a low abundance of TLR4 in the kidney cortical tubular epithelium. In addition, other members of the TLR family may fulfill the role of antimicrobial guardian in the kidney. Of particular interest in this regard is the recent work by Zhang et al., who discovered a novel receptor called TLR11 that is particularly abundant in the kidney and bladder. When infected with uropathogenic bacteria, mice deficient in TLR11 harbored at least 10,000 times as many bacteria in their kidneys as normal mice, supporting the hypothesis that TLR11 provides a barrier that prevents uropathogenic bacteria from infecting the kidneys. It appears that TLR11 activates the same general signaling pathways as other TLRs, including the involvement of adaptors MyD88 and TRAF6, stimulation of IRAK kinase, and activation of transcription factor NF-κB. At this point, however, it is unclear what that actual ligand for TLR11 might be. The similarity between TLR11 and TLR5 suggests that flagellin-like proteins, such as the one found in the pili of uropathogenic bacteria, might bind to TLR11. Interestingly, human beings have a truncated form of TLR11 that probably is inactive. Therefore, it is conceivable that the lack of functional TLR11 predisposes human beings to urinary tract infections.

**Detrimental Effects of TLR Stimulation in Kidney Disease**

A variety of bacterial products including LPS endotoxins may cause the diffuse inflammatory response seen in sepsis including acute renal failure. The release of tumor necrosis factor (TNF) after exposure to LPS in this context is thought to be the key early mediator of this syndrome. This concept is supported by studies showing that mice either deficient in TNF receptor or lacking functional TLR4 (eg, C3H/HeJ strain, see earlier) are resistant to LPS-induced acute renal failure (ARF). Furthermore, transplant studies showed that C3H/HeJ recipients of wild-type kidneys but not wild-type recipients of C3H/HeJ kidneys develop severe LPS-induced ARF. These observations are consistent with the model in which LPS acts on extrarenal TLR4, leading to the rapid release of TNF into the circulation. In turn, circulating TNF would act through renal TNFR1 to cause ARF, through a variety of mechanisms that may involve renal neutrophil infiltration and renal apoptosis. In addition, LPS injection causes limited ARF in wild-type kidneys transplanted into C3H/HeJ recipients, suggesting some role for intrarenal TLR4 in LPS-induced ARF.

In addition to ARF, LPS can induce other renal conditions such as experimental immunoglobulin (Ig)A nephropathy and antiglomerular basement membrane disease. In these experimental models, LPS potently induces chemokines, which may be central to pathogenesis by recruiting immune cells into the interstitium and glomerulus. Renal tubular epithelial cells may play a role in this process by producing chemokines after direct exposure to bacterial components. For example, both LPS and lipoprotein directly affect mouse primary renal tubular epithelial cells to express important chemokines such as monocyte chemoattractant protein-1 and regulated upon activation, normal T-cell expressed and secreted (RANTES), and these rapid responses are abolished in cells that lack TLR4 and TLR2. Based on these observations, one may postulate that in pyelonephritis, excessive stimulation of TLR-mediated chemokine production in tubular epithelial cells leads to a massive influx of inflammatory cells to the renal interstitium and the subsequent development of renal insufficiency. Consequently, the regulation of TLR function in renal tubular cells may be one of the therapeutic targets for renal disease after bacterial infection.

**TLR Stimulation and Autoimmunity**

High concentrations of LPS induce activation of specific B lymphocytes through its ability to cluster many antigen receptors. In contrast, the mechanism by which lower doses of LPS can induce B cells appears to involve the simultaneous recognition of LPS by cell-surface antigen receptors and TLR. These 2 receptors cause a synergistic signal, which provokes proliferation and production of circulating antibodies. Work by Leadbetter et al showed that the concomitant engagement of surface B-cell antigen receptor (BCR) specific for IgG and TLR9 with IgG2a-chromatin immune complexes, causes the IgG2a subclass of immunoglobulins to be recognized as if it were a pathogen and triggers T-cell–independent activation of these cells. More specifically, BCR stimulation by immune complexes in this system induce biochemical events that are necessary for B-cell activation (signal 1), and in addition increases the delivery of CpG DNA to the intracellular pool of TLR9 through autophagocytosis of ligated BCRs (signal 2).

In addition to its role in B-cell activation, TLRs also may act as transducers of the signals that induce cytokines in patients with systemic lupus erythematosus (SLE). For example, IFN-α has been associated with immune dysfunction and multiple organ involvement in SLE and its production increases as disease flares. Increases in IFNα may reflect the TLR pathway and the adjuvant-like factors mediating its induction such as CpG DNA. According to the recent reports by Means et al and Lore et al, DNA-containing complexes isolated from SLE patients can activate antigen-presenting cells called plasmacytoid dendritic cells (PDC) through the cell-surface Fc receptor for IgG called CD32. These investigators showed that CD32 facilitates internalization of SLE immune complexes into intracellular lysosomes containing TLR9, enabling TLR9 to bind CpG DNA contained in these ICs, thereby initiating a TLR9 signaling pathway. It is not unlikely, however, that immune complexes containing ligands for other TLRs, such as RNA, also may activate immune cells through similar pathways (ie, Fc receptor/TLR3). In conclusion, TLRs may contribute to the development and progression of immune complex diseases through at least 2 independent mechanisms: the BCR/TLR9 pathway that in B cells provokes the secretion of autoantibodies to nuclear antigens such as self-DNA, and the CD32/TLR9 pathway in PDCs that can mediate the production of multiple cytokines and che-
mokines that in turn play important roles in the pathogenesis of SLE. Of note, LPS is not a highly effective inducer of IFN-α. Therefore, TLR4 may not be the most likely candidate for a mediator of IFN-α-pathway activation in SLE.

Although TLR9 generally is thought to be crucial for sensing pathogens by recognizing hypomethylated CpG base pairs in bacterial DNA, these structural motifs are also found in mammalian DNA promoter elements. Therefore, it is conceivable that endogenous DNA that is released during normal or pathologic cell death stimulates B cells through the BCR/TLR9 pathway. Interestingly, the CpG dinucleotide frequency of the DNA found in SLE immune complexes is 5 to 6 times higher than the expected frequency in the genome. Alternatively, humoral disease activity in SLE might be triggered by the presence of CpG that is derived from infectious organisms during intercurrent infections.

In concordance with the role for TLR9 in the development of autoimmunity are studies on progression of lupus nephritis in MRL/lpr/lpr mice that received CpG. In these animals, both E. coli DNA and CpG oligonucleotides were associated with an increased concentration of serum anti-DNA autoantibodies, progression from mild to crescentic glomerulonephritis, interstitial fibrosis, and heavy proteinuria. Interestingly, drug chloroquine, which inhibits TLR9-mediated signaling by inhibiting acidification of endosomes to which interaction of CpG-DNA with TLR9 has been localized, is effective in some patients with SLE. Therefore, drugs that specifically target TLR signaling pathways could be promising new treatments for antibody-mediated autoimmune diseases and their complications.

TLRs and Transplant Rejection

A recent clinical study has provided evidence that TLRs contribute to alloimmunity. Work from Palmer et al has shown that TLR4 gene polymorphisms affect clinical acute lung rejection. This group showed that Asp299-Gly and Thr399-Ile mutations in TLR4 are associated with reduced immune responses to LPS and that lung transplant recipients with these TLR4 polymorphisms show a delayed time to the first acute rejection episode and a reduced incidence of acute rejection. They also found that TLR4 polymorphisms in the donor kidney had no effect on rejection. In agreement with these results are experimental data showing that minor antigen-mismatched allograft rejection cannot occur in the absence of MyD88 signaling. This could be owing to the defective maturation of dendritic cells, which require MyD88 for stimulation of IFN-γ-producing TH1 cells and expansion of graft-reactive T cells. Nevertheless, TLR-mediated allograft rejection is not completely clear because in fully major histocompatibility complex–mismatched allogenic models both skin and cardiac allografts in the absence of MyD88 were rejected without a significant delay as compared with wild-type animals. Although this study contrasts with work by Palmer et al, there are likely to be important clinical factors such as the use of generalized immunosuppression and superimposed infections that may account for these differences.

Conclusions

Since the original discovery of the Toll receptor in Drosophila and its first mammalian homolog, several thousand reports have been published on TLRs. However, our understanding of the role that distinct TLRs may play in the pathogenesis of kidney disease has just started to emerge. In particular, studies on human beings and experimental animals that lack functional TLRs provide compelling evidence that TLRs are critical defenders against microbial invasion of the urinary system. It should be remembered, however, that innate immunity, although largely effective against microbial invasion, comes at the cost of changes that promote the development of ARF during sepsis, exacerbation of autoantibody production, and propagation of inflammation and the accompanying tissue destruction in autoimmune diseases. Therefore, the development of drugs targeting TLRs should be aimed at negative consequences of TLR signaling while leaving intact components of the TLR pathway that are essential to our protection against the microbes.

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