Leptospirosis renal disease: Understanding the initiation by Toll-like receptors

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Leptospirosis is a prevalent infectious disease affecting both humans and animals worldwide. This infection is associated with occupational or recreational exposure to animals as well as contact with leptospires, particularly in flood-prone areas. Multiple organ dysfunctions may be associated with acute severe leptospirosis. A triad presentation of fever, jaundice, and acute renal failure in patients with acute multiple organ dysfunction should alert physicians to possible leptospirosis. Penicillin is effective and can rescue multiple organ failure if administered early. Renal involvement is common in leptospirosis characterized by tubulo-interstitial nephritis, and tubular dysfunction. Leptospira outer membrane proteins (OMPs) may elicit tubular injury and inflammation through Toll-like receptors (TLRs)-dependent pathway followed by activation of nuclear transcription factor kappa B and mitogen-activated protein kinases and a differential induction of chemokines and cytokines relevant to tubular inflammation. Leptospira OMP may also induce activation of the transforming growth factor-β/Smad-associated fibrosis pathway leading to accumulation of extracellular matrix. Thus, leptospirosis renal disease is a model for understanding the pathogenesis and initiation of pathogen-induced tubulo-interstitial nephritis and fibrosis. In particular, TLRs may be important mediators.

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Leptospirosis is the most widespread zoonosis, particularly in warm and humid regions.[1] The disease is caused by the spirochetal infection, leptospira-infected animals shedding urine into water or soil to infect human via skin or gastrointestinal routes. Occupational exposures, recreational activity, flooding, and household environments in close contacts with animals increase the risk of infection in humans.

A wide range of clinical manifestations occurs in humans acquiring pathogenic leptospira from animals. The clinical syndromes of leptospirosis vary from subclinical infection, self-limited anicteric febrile illness to severe and potentially fatal disease.[1,2] As a worldwide infection, endemic and epidemic spread of leptospirosis has caused morbidity and mortality, which suggest that leptospirosis is a re-emerging infectious disease throughout the world especially in developing areas.[3]

Interestingly, the kidney is the main target of leptospira in both acute and chronic infection. Acute kidney injury as a result of tubulointerstitial nephritis is an early and primary manifestation of systemic leptospirosis.[4] In chronic infection, leptospira may colonize and persist in the proximal tubule leading to carrier state and progress to chronic tubulointerstitial nephritis and fibrosis.[1] Early *in vitro* observation has revealed that pathogenic leptospira, but not non-pathogenic leptospira, attached to cultured renal epithelial cells soon after adding to culture cells.[5] These observations suggest that leptospira is a kidney-prone micro-organism, elicits tubulointerstitial nephritis and progressing to fibrosis; it is therefore an appropriate model for studying the pathogenesis of micro-organism-related kidney diseases. The pathogenic mechanism has been clarified in recent years by *in vitro* and *in vivo* models employed to dissect the initiating role of leptospirosis renal diseases. This mini-review summarizes the recent advances in understanding the initiation of renal injury and inflammation in leptospirosis and the role of innate immunity Toll-like receptors (TLRs) that could be a common initiation mechanism of kidney diseases.

CLINICAL MANIFESTATION OF LEPTOSPIROSIS

Typical clinical manifestations of leptospirosis fall into four categories: (i) mild influenza-like illness; (ii) Weil's syndrome characterized by jaundice, renal failure, hemorrhage, and

myocarditis with arrhythmias; (iii) meningitis/meningoencephalitis; and (iv) pulmonary hemorrhage with respiratory failure.[6] In 5–10% of leptospirosis cases, multiple organ damage is induced with characteristic kidney, liver, and lung lesions. Weil's syndrome, the most severe form of the infection, is characterized by febrile illness, hemorrhagic tendency, hepatic dysfunction, and acute renal failure, and may lead to fatality at a short time. A triad presentation in acute ill patients with fever, jaundice, and acute renal failure should alert the clinician of possible leptospirosis.[4,7] The presence of oliguria is an independent risk factor for mortality and non-survivors have an increased incidence of cardiac arrhythmia, dyspnea, and pulmonary rales.[8]

DIAGNOSTIC METHOD FOR LEPTOSPIROSIS

The diagnosis of leptospirosis depends mainly on serologic methods (microscopic agglutination test) to detect antibody to leptospira, polymerase chain reaction to detect leptospira DNA and culturing the micro-organism.[9] The microscopic agglutination test is the most widely used diagnostic method because of the relative difficulty in culturing leptospira. Recently, immunoglobulin M assay has been used for rapid screening of acute leptospirosis.[10]

CLINICAL DISTINCTION OF LEPTOSPIROSIS FROM MULTIPLE ORGAN DYSFUNCTION PATIENTS

Systemic infections from microorganisms other than leptospira may also induce multiple organ dysfunctions and confuse the clinical diagnosis of leptospirosis. A case–control study was conducted for differential diagnosis of leptospirosis and other infections.[11] Twenty-two confirmed and twenty-one excluded cases of leptospirosis were identified from 169 suspected cases. The most common presentations of leptospirosis group were fever (95.5%, 21/22), acute renal failure (86.4%, 19/22), myalgia (72.7%, 16/22), and jaundice (63.6%, 14/22). Incidence of hemorrhagic diathesis, myalgia, enlarged kidneys, sterile pyuria, and thrombocytopenia were increased in the confirmed leptospirosis group in comparison with those for the excluded cases. Penicillin treatment was effective in the confirmed leptospirosis group and resulted in low fatality rate of 4.5% (1/22). This case–control study provides characteristics of leptospirosis that could be identified for differentiation from other infectious diseases. Thus, prompt recognition of leptospirosis by characteristic presentations and timely antibiotics treatment can reduce the mortality rate for infected patients, even in cases with severe multiple organ damage.

ACUTE RENAL FAILURE AND THROMBOCYTOPENIA

According to a previous study,[12] thrombocytopenia is closely correlated with occurrence of acute renal failure and is described in all anicteric cases with acute renal failure.[13] Thrombocytopenia in association with acute renal failure appears independently of disseminated intravascular coagulation and may be concurrent with severe endotoxin injury of leptospirosis.[14] In a series study of 12 patients with acute renal failure, thrombocytopenia occurred in eight patients and, notably, in four patients requiring hemodialysis.[14] Therefore, thrombocytopenia may be an associated sign of acute leptospirosis renal disease.

ACUTE TUBULOINTERSTITIAL NEPHRITIS AND TUBULAR TRANSPORT DYSFUNCTION

Renal involvement is common in leptospirosis. Renal dysfunction ranges from prerenal azotemia to severe acute renal failure requiring dialysis therapy. The incidence of acute renal failure varies widely reaching as high as 40–60% in severe leptospirosis.[15] Hypokalemia occurs frequently due to tubular dysfunction and tubulo-interstitial nephritis is the principle renal lesion to cause renal failure. In addition, severe hypotension is an important sign for subsequent development of renal and pulmonary complications.[16] Thus, hemodynamic alterations, immune response, and direct nephrotoxicity are involved in the development of renal lesions.

Tubulointerstitial nephritis with interstitial edema and mononuclear cellular infiltration are main pathologic findings.[17,18] Glomerular changes are usually unremarkable. Tubular cell necrosis and vasculitis are observed in the acute phase of the disease. Previous reports have shown that the main factors in the pathogenesis of the renal lesions are related to the presence of micro-organisms elicited by their migration, elaboration of their virulent toxins, and the induction of immune response.[19] In the infected host, pathogenic leptospires can disseminate hematogenously and invade kidneys, which represent one of the most frequently affected organs. Through the bacterial invasion and toxicity of outer membrane components with generation of cytokines, chemokines, and cellular infiltration lead to the development of tubulointerstitial nephritis.[1,4,20] Clinically, non-oligouric acute renal failure, hypokalemia, and sodium wasting occur frequently in leptospirosis. The pathophysiology of tubular dysfunction in leptospirosis involves proximal tubular dysfunction, augmenting distal sodium delivery, and, consequently, potassium excretion by the intact distal tubule.[21]

In *Leptospira santarosai* serovar Shermani infected patients presenting with polyuria and hypokalemia, detailed *in vivo* tubular clearance tests were performed and identified a defective Na^+-K^+-Cl^- co-transporter (NKCC2) and poor response to furosemide infusion due to tubular dysfunction.[22] An *in vitro* study using medullary thick ascending limb-cultured cells derived from normal mice, demonstrated downregulation of NKCC2 mRNA expression and inhibition of NKCC2 co-transporter activity in medullary thick ascending limb cells by *Leptospira santarosai* serovar Shermani outer membrane protein (OMP) extract. Effective treatment of leptospirosis reverses the tubular dysfunction.[23] These changes may account for the observed electrolyte disorders induced by *Leptospira santarosai* serovar Shermani.[24] However, the changes may not be a general phenomenon, as other serovars such as *Leptospira interrogans*

serovar Bratislava did not affect function of NKCC2[24] and the levels of NKCC2 may be increased in the hamster model induced by *Leptospira pomona* serovar Pomona.[25]

CHARACTERISTIC SONOGRAPHIC FINDINGS

Characteristic early renal sonographic findings in acute leptospirosis are swollen kidneys and relatively normal parenchymal echogenecity indicating tubulointerstitial edema.[4] The enlarged kidney returns to a normal size after effective treatment of the acute leptospirosis.[11]

REDUCED MORTALITY WITH PENICILLIN TREATMENT

Clinical studies have demonstrated the effectiveness of early antibiotic treatment for treating leptospirosis.[26] Intravenous penicillin is recommended in severe cases, but oral antibiotics such as doxycycline, amoxycillin, ampicillin, or erythromycin are effective in less severe cases. Third-generation cephalosporins such as ceftriaxone also appear to be effective.[27,28] Jarisch–Herxheimer reactions may occur following effective treatment. Although leptospira has shown sensitivity to a wide range of antibiotics in *in vitro* and animal experiments, clinical experience with the newer antibiotics is limited. Early intravenous administration of penicillin has proven effective in leptospirosis patients. In a series, serum creatinine levels of confirmed cases improved in a mean of 2.7 ± 2.2 days after intravenous penicillin administration.[11] Therefore, treatment with effective antibiotics should be initiated as soon as leptospirosis is suspected pending confirmation.

UNDERSTANDING THE PATHOGENESIS OF LEPTOSPIROSIS RENAL DISEASE

The mechanism of leptospirosis renal dysfunction has not been fully studied and elucidated despite being an important cause of acute renal failure worldwide. Early observational studies have indicated that the leptospira spread through hematogenous route to the kidney, circulate to glomeruli, peritubular capillary and migrate to interstitium, renal tubule, and finally remain in the proximal tubular lumen.[20] Similar renal injuries have been reproduced in animal models of leptospirosis. The kidneys of guinea pigs with leptospirosis revealed tubular cell injury, interstitial nephritis and associated microvascular injury, particularly at the corticomedullary junction.[29,30] Ultrastructural study of the kidney after inoculation of leptospira in mice demonstrated the entry route of leptospira is by penetration of the capillary lumen at day 2 followed by entrance into interstitial tissue elaborating edema and cellular infiltration at days 4–8. Leptospira can be found in the proximal tubular cells at day 10, and in the tubular lumen at day 14.[31] Leptospiral antigens are found in the proximal tubule cells, macrophages, and in the form of large extracellular clumps in the interstitium.[32] A leptospiral glycolipoprotein, a component of leptospiral endotoxin, has been found expressed in infected renal tubules and vascular lumen of the interstitium, which parallels tubulo-interstitial nephritic changes.[30] The association of leptospira with membrane-bound protein droplets in proximal tubules may thus be important in the induction of tubulo-interstitial nephritis and of chronic carrier state in rat leptospirosis where diffuse interstitial nephritis and fibrosis may be initiated.[33]

LEPTOSPIRA OMP AND ENDOTOXINS

The outer membrane of spirochete and gram-negative bacteria serves as a permeability barrier. In leptospira, the outer membrane contains antigenic and virulent components including lipoprotein, lipopolysaccharide, and peptidoglycan. Clinical and pathological observations suggest that outer membrane components may play a role in the pathogenesis of leptospirosis. Leptospiral endotoxins, located on the outer membrane, appear to be the major antigens affecting immunity to leptospira and might be responsible for renal dysfunction.[34] Owing to their location on the cell surface, leptospira OMPs are likely to be relevant to host–pathogen interactions determining virulence and pathogenesis. Immuno-electron microscopy has revealed leptospiral antigen adjacent to cell membrane of hepatocyte, kidney tubular cells and endothelial cells of the interstitial capillary in animal study.[35] A disturbed tubular function and inflammation, therefore, may be elicited by the leptospiral OMP components. Several OMP of pathogenic leptospira have been identified and localized to proximal tubules and the interstitium in infected animals.[36,37] To elucidate the mechanism of tubulo-interstitial injury caused by leptospira infection, the effect of the leptospira OMP extract on cultured mouse renal tubular epithelial cells on the expression of a variety of genes related to tubular injury and inflammation was evaluated.[38] The leptospira OMP activates nuclear transcription factor kappa B (NF-κB), activator protein-1, and downstream genes expressed in medullary thick ascending limb cells.[38] The OMP is heat labile and an antiserum raised against leptospira may prevent these effects. Further identification of LipL32, a major pathogenic outer membrane lipoprotein, induces tubulointerstitial nephritis-mediated gene expression in mouse proximal tubule cells.[39] Previous studies have demonstrated that LipL32 expresses during cultivation and mammalian infection. Immunohistochemistry has confirmed intense LipL32 reactivity with leptospira infecting proximal tubules of hamster kidneys and as a prominent immunogen during human leptospirosis.[40] LipL32 is also a hemolysin, which causes hemolysis of erythrocyte during leptospira infection.[41,42] The sequence and expression of LipL32 is highly conserved among pathogenic leptospira species indicating that LipL32 may be important in the pathogenesis, diagnosis, and prevention of leptospirosis.

In the kidney, LipL32 directly affects proximal tubular cells by substantially increasing gene and protein expression of proinflammatory cytokines such as inducible nitric oxide (iNOS), monocyte chemoattractant protein-1 (CCL2/MCP-1), regulated upon activation, normal T-cell expressed and secreted (RANTES), and tumor necrosis factor (TNF-α). Increased nitric oxide expression by medullary thick ascend-

ing limb cell suggests that hemodynamic adaptation of the protective vasodilatation mechanism is being elicited. However, an injurious condition may be induced by production of peroxynitrite and free oxygen radicals. The chemokine CCL2/MCP-1 is involved in interstitial nephritis, one of the most important initiating factors in monocytic cells infiltration in interstitial nephritis. The increase of CCL2/MCP-1 may indicate the role of OMP in tubulo-interstitial nephritis. The cytokine TNF-α, an immune and inflammatory cytokine that mediates enodotoxemia, may affect inflammation in leptospirosis renal disease. These findings indicate the importance of LipL32 in the pathogenesis of leptospirosis renal diseases. Therefore, identification of novel OMP of the leptospira shall continue as a primary focus for future study for the pathogenesis, diagnosis, and vaccine development.

Conversely, OMP extract from avirulent non-pathogenic *Leptospira biflexa* serovar Patoc did not induce significant changes in gene expression. These findings are compatible with the notion that saprophytic avirulent leptospira does not induce clinical manifestation as demonstrated by lacking characteristic leptospira endotoxin, a different pattern of outer membrane antigens expression by immunoblotting assay,[43] and readily phagocytosed by human monocyte and polymorphonuclear cells.[44,45]

TLR PATHWAYS ARE INVOLVED IN LEPTOSPIROSIS TUBULO-INTERSTITIAL NEPHRITIS

TLRs are proteins that recognize specific molecular patterns of pathogens representing the first line of defence in innate immunity. These receptors interact with a variety of exogenous and endogenous ligands and initiate a cascade of signaling events leading to the production of numerous cytokines and effector molecules. The effect of TLRs was evaluated to determine whether TLRs could mediate the inflammatory response induced by leptospiral OMP in renal proximal tubule cells.

In the cultured renal proximal tubule cells, *Leptospira santarosai* serovar Shermani OMPs and LipL32 induced a significantly increased expression of TLR2 but not TLR4. The increased expression of iNOS and CCL2/MCP-1 mRNA expressions could be suppressed by an anti-TLR2 antibody, but not by an anti-TLR4 antibody. Furthermore, leptospiral OMP stimulated both CCL2/MCP-1 mRNA and secreted protein in transfected human embryonic kidney 293 cells with a TLR2-expressing plasmid but had no effect on cells with a TLR4-expressing plasmid. These findings signify that the stimulation of iNOS and CCL2/MCP-1 caused by pathogenic leptospiral OMP, in particular LipL32, in proximal tubule cells requires TLR2 for the early inflammatory response.[46]

DIFFERENTIAL INDUCTION OF TLR2-ASSOCIATED SIGNALS IN PROXIMAL TUBULE CELLS

Further study of the TLRs signaling pathway showed that *Leptospira santarosai* serovar Shermani OMP stimulated the secretion of pro-inflammatory mediators in renal tubule epithelial cells in a differential induction manner dependent on mitogen-activated protein kinase (MAPK) and NF-κB associated pathways. First of all, the leptospira OMP upregulated TLR2 mRNA expression in proximal tubule cells in a time- and dose-dependent effect. Further, leptospira OMP stimulated the activation of extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNKs), and p38 MAPK, initiated the NF-κB, activator protein-1 and enhanced the secretion of CCL2/MCP-1 and CXCL2/MIP-2. The OMP enhanced the secretion of CCL2/MCP-1 and CXCL8/interleukin-8 (CXCL8/IL-8) in TLR-defective human embryonic kidney 293 cells only when transfected with a TLR2 expressing plasmid. Secretions of CCL2/MCP-1 and CXCL2/MIP-2 stimulated by OMP were significantly reduced by incubating proximal tubule cells with p38 MAPK inhibitor. Finally, a neutralizing anti-mouse TLR2 antibody hindered the phosphorylation of p38 and OMP-stimulated secretion of CCL2/MCP-1 and CXCL2/MIP-2. These findings demonstrate that activation of p38 MAPK and release of chemokines by OMP are mediated by TLR2 in renal proximal tubule cells.[47]

Cytokine protein array analysis has further revealed significant upregulation of neutrophil-chemoattractant keratinocyte-derived chemokine (CXCL1/KC) at nanogram range of OMP stimulation in cultured murine proximal tubule cells. The OMP stimulated early secretion of human growth-related oncogene-α, a functional homolog to murine KC, in TLR-defective human embryonic kidney 293 cells transiently transfected with TLR2-expressing plasmids and the response was augmented by coexpression of TLR1 and TLR2. Moreover, silencing of TLR2, myeloid differentiation factor 88, and TNF receptor-associated factor 6 with specific small interfering RNA significantly reduces the response caused by OMP in proximal tubule cells. The OMP stimulated CXCL1/KC secretion was also significantly reduced by pre-incubating proximal tubule cells with a specific p38 inhibitor. These results indicate that OMP stimulates the production of CXCL1/KC to recruit polymorphonuclear neutrophils at the site of inflammation through a TLR2-mediated pathway in renal tubule cells. Thus, the leptospira OMP stimulated the phosphorylation of three MAPK extracellular signal-regulated kinase, c-Jun N-terminal kinase, and P38 and the increase of CXCL2/MCP-1, CCL2/MIP-2, and CXCL1/KC was associated with P38 pathway.[48] Further inhibitor experiments showed that the NF-κB pathway is associated with increased iNOS and TNF-α in the proximal tubule cells. These experimental results indicated that the two major pathways associated with TLR2 signaling elaborated differential induction of different cytokines or chemoattractants molecules responsible for early inflammation. These results also implicate the crucial role of innate immunity in leptospira-induced tubulointerstitial nephritis (Figure 1).

By applying the same infection model to other organ systems, a recent study has confirmed that microglial cells respond to pathogenic but not non-pathogenic Leptospira

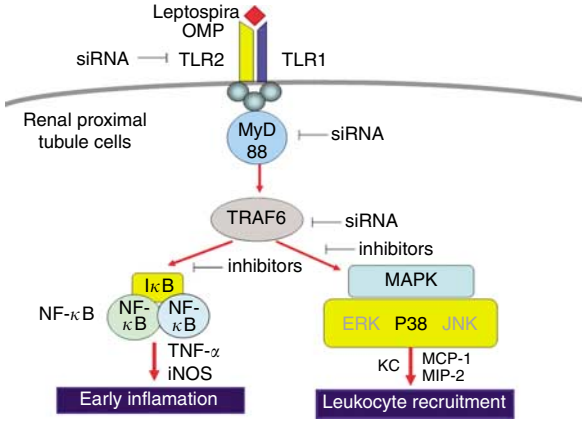

Figure 1 | Differential induction of early inflammatory genes via TLR2 Pathway by leptospira OMP in renal proximal tubule cells. NF-κB and MAPK pathways can be activated and be inhibited by siRNA and inhibitors for each component and pathway.

infection with a time- and dose-dependent induction of molecular signals including p38 phosphorylation and NF-κB activation. This response is followed by the production of soluble factors such as cytokines and nitric oxide. The phenomenon is reproduced by leptospiral lipoproteins and, to a lesser extent, by leptospiral-derived lipopolysaccharide.[49] This study confirmed that a similar pathway of leptospira cellular effects on different organ systems may exist and that the leptospira OMP component has a relatively greater importance than the leptospira lipopolysaccharide in eliciting organ damages.

TLRS AND LEPTOSPIROSIS

Leptospiral outer membrane constituents were shown to activate macrophages through TLR2 pathway.[50] Leptospira lipopolysaccharide constitutes the predominant signaling component for macrophages through this pathway. Conversely, TLR4 is apparently not involved in cellular responses to leptospira. Subsequently, TLR4 is known to be crucial for protection from acute lethal infection and control of leptospiral burden during sublethal chronic leptospira infection in mice. Studies of a murine model, which directly recapitulates severe human leptospirosis, suggest a critical role of TLR4 gene or its associated components, in early infection by leptospira.[51] Intact TLR4 signaling contributes to the control of the tissue burden of Leptospira in non-lethal infection. Studies of TLR4-deficient mice have revealed significantly higher numbers of leptospires, particularly in the target organs such as the liver, and the lung for mediating leptospiral disease and in kidney that both mediating and transmitting leptospira. Macrophages from TLR4-deficient mice secreted far-lower levels of cytokines than wild-type macrophages in response to leptospira. The key finding was that intact TLR4 function was critical for mouse survival and for preventing jaundice, pulmonary hemorrhage, and death. Thus, the TLR4 pathway may offer protection from severe leptospirosis. Given the newly discovered role of TLR4 in protection from lethal infection and the known role of TLR2 in the renal tubule in initiating inflammation, different components of leptospira may activate different TLR pathways for a range of immune responses including inflammation, clearance, and protection. The responses may be diverse in trend and in intensity in a number of organ systems dependent on dissimilar TLRs.

In this context, experimental models using whole body TLR-deficiency (knock-out model) or single organ TLR-deficiency study (*in vitro* cell culture study) should be interpreted with caution. Systemic or local effects on organ damage may have dissimilar outcomes and may be complex for interpreting the effect of TLRs.[52] The effect could be protective at local level of TLR-deficient status from stimuli; on the contrary, the effect could be detrimental at systemic stimulation. For instance, systemic infection in the TLR deficiency model, in which first line defence mechanism is disrupted, may be detrimental to the target organ rather than protective, in contrast to study at local deficiency levels in the cell culture system. Other examples are that in a sepsis model and in a leptospirosis model, the outcome may differ when systemic infection or local infection is induced. Thus, to accurately interpret the role of TLR, one must realize the threshold and level of pathogen–host immune responses to determine and understand whether the protective or harmful effect is due to innate TLR immunity. The experimental results of these studies highlight the need for caution in the assessment of disease paradigms.

LEPTOSPIROSIS AND RENAL FIBROSIS

The consequence of tubulointerstitial nephritis caused by leptospiral infection is tubular atrophy and interstitial fibrosis, if chronic leptospiral infection remain untreated.[33] In canine and rat leptospirosis, the disease may undergo a chronic course, leading to tubulointerstitial fibrosis and thus chronic renal failure.[33,53] Human biopsy studies have demonstrated an association among infection of leptospirosis, interstitial nephritis, and fibrosis.[18] A recent report further indicated that leptospirosis led to irreversible tubulointerstitial fibrosis in a young male, who thus required chronic hemodialysis.[54]

To elucidate the crucial association between leptospirosis and renal fibrosis, the effect of OMP from pathogenic leptospira on extracellular matrix accumulation has been examined.[55] Adding *Leptospira santarosai* serovar Shermani OMP to proximal tubular cells, HK-2 cells, dose-dependently led to an increase of type I and type IV collagens both at mRNA and protein levels. The active transforming growth factor-β1 (TGF-β1) secretion was increased twofolds following an addition of leptospira OMP. Addition of anti-TGF-β1 neutralizing antibodies attenuated the increased production of type I and type IV collagen, indicating the participation of TGF-β1 in this process. These observations were further confirmed by the increased nuclear translocation of Smad3 after administration of leptospira OMP, and overexpression of the dominant-negative Smad3 prevented the leptospira

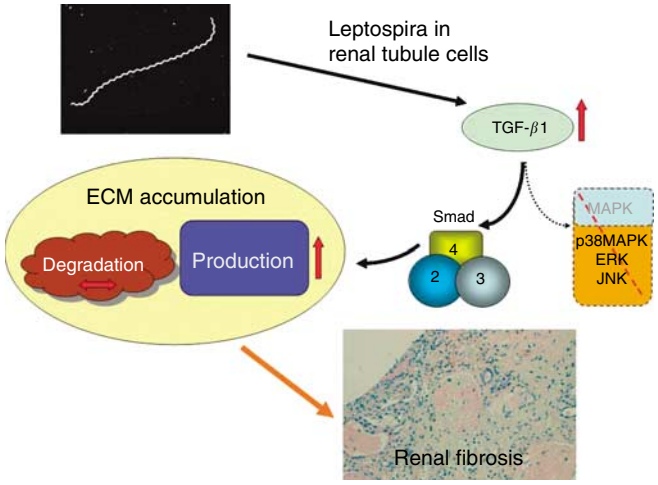

Figure 2 | Leptospira induces fibrosis pathway in the tubule cells by activating TGF-β1/Smad pathway but not MAPK. The activation results in increased production of extracellular matrix in the renal tubule cells. These activations may lead to renal fibrosis in leptospirosis patients.

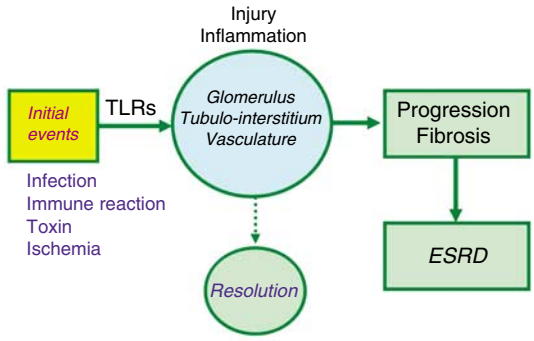

Figure 3 | The possible role of TLRs in initiation of kidney diseases. TLRs may be involved in the initiation of diverse renal diseases in response to exogenous and endogenous stimuli. On one hand, TLRs are the first line of defence for innate immune protection. Conversely, TLRs may act as a 'danger signal' by triggering inflammatory responses to stimuli when tailored immune reactions cannot adequately resolve the inflammation. The outcome may thus depend on a balanced or unbalanced immune reaction leading to either resolution or disease progression to renal fibrosis and end-stage renal disease.

OMP-induced increase of type I or type IV collagen production. On the other hand, adding the OMP had no effect on metalloproteinase activity. Therefore, the degradation pathway may not be altered. In summary, this study clearly demonstrated the stimulatory effect of leptospira OMP in enhancing extracellular matrix synthesis, which was mediated by a TGF-β1/Smad-dependent pathway (Figure 2).

In this study, administering the OMP from *Leptospira santarosai* serovar Shermani induced increased production of type IV collagen, whereas addition of the OMP from a non-pathogenic leptospira, *Leptospira biflexa* serovar Patoc, did not alter production of this collagen. These findings were compatible with our previous report demonstrating that pathogenic leptospira, but not non-pathogenic leptospira, is able to promote expression of iNOS, MCP-1, and TNF-α in tubulointerstitial nephritis. In leptospirosis patients, kidney biopsies have revealed increased expression of collagen I, IV, TGF-β, and Smad in the tubulointerstitial area, which is *in vivo* evidence of leptospirosis renal fibrosis (manuscript in preparation). Overall, the most important finding of this study is that pathogenic leptospira may induce renal fibrosis. Whether the fibrosis pathway is directly or indirectly related to activation of TLRs merits further study.

TLRS AND KIDNEY DISEASES

Available information indicates that renal TLRs may respond to exogenous and endogenous ligands, and interact between innate and adaptive immunity,[56] thereby influencing kidney function in health and disease. The renal TLRs are known to be involved in pathogenesis of glomerulonephritis[57–61] in ischemia/reperfusion renal injury;[62] sepsis-induced acute renal failure;[63] and transplant kidney rejection in favor of TLR4 polymorphism.[64,65] Experimental models have indicated TLR deficiency may protect the renal injury.[66,67] Therefore, TLRs may be involved in the initiation of diverse renal diseases in response to different exogenous and endogenous stimuli (Figure 3). On one hand, TLRs are the first line of defence for innate immune protection. Conversely, TLRs may act as a 'danger signal' by triggering inflammatory responses to stimuli when tailored immune reactions cannot adequately resolve the inflammation. The outcome may thus depend on a balanced or unbalanced immune reaction leading to either health or disease. Based on the study of isolated cell populations, *in vitro* or *in vivo* studies of knockout animals involving genetic models should cautiously interpret the final outcome and systemic effects.

CONCLUSION AND PERSPECTIVE

The role of TLRs in initiation of kidney disease represents an expanding and exciting area in the study of renal disease initiation. In the study of kidney disease pathogenesis, leptospirosis renal disease may serve to better understand the initiation of micro-organism-related kidney diseases. Recent research in leptospirosis renal disease suggests that pathogenic leptospira initiates tubulointerstitial nephritis through outer membrane components, which binds to TLR2 in renal tubule cells. The binding of the receptor is followed by activation of signaling pathways related to inflammation that are induced differentially according to different pathways. With more efficient approaches by systems biology, other novel activation pathways can be expected and understood in the future. More interestingly, the induction of renal fibrosis by leptospira indicates the possibility of pathogen-induced chronic renal diseases; understanding the initiation of these diseases is therefore critical. These findings offer a basis for understanding the molecular mechanisms controlling the innate immune response caused by leptospirosis in renal tubule cells that will serve for future studies of the pathogenesis of micro-

organism-induced tubulointerstitial nephritis and initiation of kidney diseases. In particular, TLRs may be important mediators.

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