Microbial nucleic acids pay a Toll in kidney disease

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Pawar, Rahul D., Prashant S. Patole, Markus Wörnle, and Hans-Joachim Anders. Microbial nucleic acids pay a Toll in kidney disease. Am J Physiol Renal Physiol 291: F509–F516, 2006; doi:10.1152/ajprenal.00453.2005.—Nucleic acids provide more than the genetic code that determines the morphological and functional phenotype of microbes and eukaryotes. In fact, nucleic acids have immunomodulatory functions as they are recognized by a set of pattern-recognition receptors that initiate and modulate immune responses in the host. Toll-like receptor (TLR)-3 recognizes double-stranded RNA, TLR7 and TLR8 recognize single-stranded RNA, CpG-DNA is a ligand for TLR9, and all of these TLRs are expressed in the nephritic kidney. In this review, we summarize recent advances in this field and discuss new hypotheses for the pathogenesis of kidney diseases that are triggered by infectious organisms.

Toll-like receptors; glomerulonephritis; lupus nephritis

ONLY A LIMITED NUMBER OF PATHOGENS directly invade the kidney, but hepatitis virus-associated cryoglobulinemic glomerulonephritis or sepsis represent just two examples of how antimicrobial immunity can trigger kidney disease. In fact, disease activity of many other common kidney diseases are linked to episodes of infection, including IgA nephropathy, renal vasculitis, transplant rejection, lupus nephritis, and postinfectious glomerulonephritis (Table 1). The molecular mechanisms that link antimicrobial immunity to renal dysfunction are largely unknown but are thought to be somehow related to the immunostimulatory effects of microbial antigens and the humoral consequences of a systemic inflammatory response.

Recent breakthroughs in the field of antimicrobial immunity discovered how eukaryotes recognize their microbial environment and how they balance commensalism and antimicrobial defense (42). Eleven years after its first description, the gene Toll was identified by Jules Hoffmann’s group (43) in Strasbourg to be involved in antifungal immunity of Drosophila. In the search for human homologs to Toll, a new family of pattern-recognition receptors was discovered that can recognize a great variety of pathogens, including viruses, fungi, and bacteria. The members of the Toll-like receptor (TLR) family recognize conserved molecular patterns, including peptidoglycans, lipopolysaccharides (LPS), and, most interestingly, nucleic acids (Fig. 1). A subgroup of the 11 known human TLRs recognizes various forms of microbial nucleic acids. This is not surprising, because microbial nucleic acids represent a uniform molecular pattern, allowing recognition independently of continuous evolutionary changes to the outer membrane or capsid components. A detailed review of the entire TLR family and their potential roles in kidney disease has been presented elsewhere (4).

In this review, we focus on new data concerning the subfamily of the nucleic acid-specific TLRs in view of their expression in the kidney and their functional role in glomerulonephritis. Based on these data, we discuss new hypotheses on the pathogenesis of kidney diseases that are associated with microbial infection and provide a future perspective for this field.

MICROBIAL NUCLEIC ACIDS PROVIDE A PATHOGEN-ASSOCIATED MOLECULAR PATTERN TO BE RECOGNIZED BY TOLL-LIKE RECEPTORS

Microbial nucleic acids occur in all types of pathogens and allow pathogen recognition independently of their individual phenotype or antigens. In fact, it is now believed that the immunostimulatory potential of well-known adjuvants, e.g., BCG or Freund’s adjuvant, largely relates to nucleic acid sequence motifs in these preparations (38). An ancient subgroup of the TLRs induces innate antimicrobial immunity on exposure to their nucleic acid ligands (1, 11, 15, 20, 62). Microbial nucleic acids appear as potent immune adjuvants, particularly when processed together with microbe-specific antigens (28, 68, 74). Four of 11 human TLRs specifically recognize nucleic acids (Fig. 1). TLR3 recognizes double-stranded RNA (dsRNA), which occurs in some dsRNA viruses (2). TLR7 and TLR8 recognize single-stranded viral RNA (ssRNA) (26), and TLR9 recognizes unmethylated cytosine-guanosine dinucleotide (CpG) motifs in ss- or dsDNA that may originate from viruses or bacteria (30).

Compartment-Specific Localization

The most striking difference between nucleic acid-specific TLRs and the other TLRs is their localization within an intracellular compartment, which may contribute to the discriminating between self and nonself (Fig. 1) (8). For example, in dendritic cells and mouse macrophages, TLR9 resides in the endoplasmatic reticulum (40). On intracellular uptake of CpG-DNA into early endosomes, TLR9 redistributes from the endoplasmatic reticulum to CpG-DNA-containing endosomes (40). Chloroquine and other inhibitors of endosomal acidification prevent signaling through TLR3, TLR7/8, and TLR9, which argues for endosomal maturation as a critical step in this process (17). Thus nucleic acids that find their way into the
Table 1. Human kidney diseases that relate to infections

<table>
<thead>
<tr>
<th>Glomerulonephritis (GN)</th>
<th>Disease Flares Related to Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postinfectious GN</td>
<td>IgA nephropathy</td>
</tr>
<tr>
<td>Cryoglobulin-related GN</td>
<td>Renal vasculitis</td>
</tr>
<tr>
<td>Anti-GBM disease</td>
<td>Lupus nephritis</td>
</tr>
<tr>
<td>HIV-associated nephropathy</td>
<td></td>
</tr>
<tr>
<td>Membranous GN (occasionally)</td>
<td></td>
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<tr>
<td>Membranoproliferative GN (occasionally)</td>
<td></td>
</tr>
</tbody>
</table>

Tubulointerstitial disease

| Septic renal failure     | Chronic renal failure             |
| Infective pyelonephritis | Chronic allograft dysfunction     |
| Hantavirus infection     |                                   |
| Acute allograft rejection|                                   |
| Cytomegalovirus infection|                                   |
| Polyomavirus infection   |                                   |

Cell Type-Specific Expression

In mice and humans, TLR3 is expressed by myeloid dendritic cells, but mice also express TLR3 on monocytes (2, 6, 27). TLR3 is the only nucleic acid-specific TLR expressed by nonimmune cells, e.g., glomerular mesangial cells in mice and humans (54, 73, 80) and human vascular endothelial cells (71).

Signaling

All TLRs share a common cytoplasmic signaling domain, the Toll-interleukin 1 (IL-1) receptor (TIR) domain. The TIR domain can specifically bind to a family of TIR domain-containing adaptors that are linked to a set of different signaling pathways (Fig. 1) (1). TLR3 signaling specifically involves the TIR domain-containing adaptor protein-inducing interferon-β (TRIF) (82). TLR3 signaling is entirely TRIF dependent (2, 81). The TLR3 signaling pathways downstream of TRIF are threefold: activation of the transcription factors AP-1 and NF-κB induce expression of many proinflammatory genes (50, 82); activation of the transcription factors IRF-3 and -7 results in expression of type I interferons (81); and the serine-threonine kinase RIP3 triggers caspases-induced apoptosis (23).

Biological Effects

In mesangial cells, at least two of these TLR3 signaling pathways can be activated. TLR3 activation on mesangial cells induces multiple proinflammatory mediators all regulated by the transcription factors AP-1 and NF-κB. The IRF-3 pathway seems not to be relevant in mesangial cells as these cells do not generate type I interferons. The apoptosis pathway of TLR3 signaling can be activated in mesangial cells as TLR3 ligation induced mesangial cell apoptosis in vitro and in vivo (54, 80). TLR ligation on immature dendritic cells is followed by activation and maturation to the antigen-presenting phenotype, and migration to lymphoid tissues. Dendritic cells produce selected cytokines in response to pI:C RNA, e.g., IL-12, which affects T cell priming toward a Th1 phenotype (2, 34). TLR3 ligation stimulates dendritic cells to produce large amounts of type I interferons, which play a dominant role in the innate immune response during viral infection (48, 69).

TLR7 AND -8

TLR7 and -8 are close relatives arising from a recent X-linked duplication event (19). TLR7 and -8 were first identified to recognize a group of synthetic imidazoquinolines (29). The natural ligand for human TLR7 is GU-rich single-stranded viral RNA (17, 26, 46, 52). A biological function of TLR8 in mice is still questionable, because phagosome may be detected by these TLRs and subsequently elicit immune responses. However, different types of nucleic acids induce different types of immune responses due to different signaling pathways of TLR3, TLR7/8, and TLR9 as well as due to cell type-specific expression of these TLRs.

TLR3

The ligand for TLR3 is dsRNA, e.g., released from dsRNA viruses during infection (2). It has been reported that endogenous mRNAs or small-interference RNAs ligate TLR3 in vitro, but the significance of this finding in vivo is unclear at present (9, 12, 35, 36).

Cell Type-Specific Expression

In mice and humans, TLR3 is expressed by myeloid dendritic cells, but mice also express TLR3 on monocytes (2, 6, 27). TLR3 is the only nucleic acid-specific TLR expressed by nonimmune cells, e.g., glomerular mesangial cells in mice and humans (54, 73, 80) and human vascular endothelial cells (71).

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Biological Effects

In mesangial cells, at least two of these TLR3 signaling pathways can be activated. TLR3 activation on mesangial cells
TLR7-deficient mice respond to neither imidazoquinolines nor single-stranded viral RNA.

**Expression on Cells**

TLR7 is expressed on both human and mouse plasmacytoid and myeloid dendritic cells as well as B cells (21, 29). TLR8 is only found on human myeloid dendritic cells and macrophages (31). TLR7 and -8 are apparently absent on nonimmune cell types.

**Signaling**

The interaction of TLR7/8 with their ligand leads to selective recruitment of the adaptor molecule MyD88 from the cytosol to the outer endosomal membrane, where it interacts with the intracellular TIR domain of TLR7/8 (Fig. 1). Through activating IRAK4 and TRAF6, TLR7/8 signaling finally activates NF-kB, which induces many proinflammatory genes including TNF-α, IL-6, IL-1β, IL-8, IL-12, IL-18, as well as chemokines (13, 57).

**Biological Effects**

Imiquimod and resiquimod were found to activate dendritic cells, macrophages, and B cells in mice to produce type I interferons, IL-12, and TNF-α in vitro (26, 29) and in the human skin (61). This specific cytokine response enhances antiviral immunity also against DNA viruses such as cytomegalovirus and human immunodeficiency virus (45). This can be explained by an immunostimulatory adjuvant effect of TLR7 ligands on antigen presentation and subsequent T cell priming by dendritic cells (16, 45, 65).

**TLR9**

The ligand for TLR9 is CpG-DNA, a dinucleotide sequence that has been identified as a stimulatory motif of bacterial and viral DNA (30, 38, 39). By using synthetic CpG-oligodeoxyribonucleotides (ODN), distinct nucleotide sequences have now been identified that provide maximal stimulation or even inhibitory effects in cells of either human or murine origin (38, 44).

**Cell Type-Specific Expression**

In contrast to TLR3 but similar to TLR7 and TLR8, TLR9 is not expressed on nonimmune cells. Among the different human immune cell populations, TLR9 expression is restricted to B cells and to plasmacytoid dendritic cells, whereas in mice TLR9 is also expressed on monocyte/macrophages and myeloid dendritic cells (75).

**Signaling**

Similar to TLR7/8, the interaction of TLR9 with CpG-DNA leads to recruitment of MyD88 from the cytosol to the outer endosomal membrane, where it interacts with the intracellular TIR domain of TLR9 (40). The TLR9-dependent expression of proinflammatory cytokines and chemokines is induced through the same intracellular signaling pathway used by TLR7/8.

**Biological Effects**

CpG-DNA is a strong B cell mitogen (39). For example, immune complexes that contain CpG-DNA have been demonstrated to induce T cell-independent B cell activation, proliferation, and autoantibody production by cross-linking Fc receptors with TLR9 (41). In fact, linkage of CpG-DNA to an antigen provides a powerful signal for the activation of other antigen-presenting cells, including dendritic cells and, in mice, macrophages (75). Interaction of CpG-DNA-with TLR9 induces antigen-presenting cells to secrete Th1 cytokines, i.e., IL-12 or IFN-γ (24). Subsequent T cell or B cell responses are modulated toward a Th1-type response, which can be therapeutic in Th2-type diseases like asthma and atopy (74, 76) but that may aggravate other types of chronic autoimmune tissue injury, e.g., arthritis, encephalitis, or glomerulonephritis (3, 32, 51). Furthermore, at high doses CpG-DNA-induced activation of TLR9 can lead to toxic shock (30). Repeated injections with CpG-DNA cause hepatotoxicity, systemic cytokine release, lymphoproliferation, and functional immunosuppression (25). In summary, microbial DNA, as well as other types of microbial nucleic acids, appears to have potent immunomodulatory functions that may well affect immune-mediated diseases of the kidney.

**MICROBIAL NUCLEIC ACIDS IN THE PATHOGENESIS OF KIDNEY DISEASES**

**Glomerulonephritis**

Postinfectious glomerulonephritis is a paradigmatic disease that links glomerular injury to circulating immune complexes containing bacterial products. Also, chronic hepatitis C and human immunodeficiency virus (HIV) infection can be complicated by glomerulonephritis via this mechanism. Disease flares of IgA glomerulonephritis may also be associated with viral infection. Several lines of evidence support the hypothesis that viral RNA may be involved in that process. First, injected viral dsRNA distributes to the glomerulus and is taken up by mesangial cells into intracellular endosomes in mice with glomerulonephritis (54). Second, as mentioned above, cultured murine and human mesangial cells produce IL-6, IL-1β, IL-8, ICAM, M-CSF, and other proinflammatory factors on uptake of viral dsRNA (54, 80). PCR analysis of microdissected glomeruli from patients with hepatitis C-positive glomerulonephritis showed increased TLR3 mRNA expression compared with glomerular RNA isolates from normal human kidney (80). Thus circulating viral dsRNA was identified as a possible mechanism of mesangial cell activation in glomerulonephritis (Fig. 2). Interestingly, mesangial cells do not express TLR7/8 and TLR9, which should not allow activation of mesangial cells by nucleic acids from DNA and ssRNA viruses. In fact, none of the DNA viruses are commonly associated with glomerulonephritis (Table 2). However, among the RNA viruses rather ssRNA viruses, e.g., hepatitis C virus and HIV, than dsRNA viruses, e.g., reovirus and rotavirus, are associated with glomerulonephritis. Whether double-stranded or hairpin-like transcripts of ssRNA viruses activate TLR3 on mesangial cells is unknown to date. This is not unlikely to happen in vivo because lethal infection with the ssRNA West Nile virus was found to be markedly attenuated in TLR3-deficient mice (77). Therefore, it is hypothesized that circulating viral RNA, usually complexed in immune complexes that provide resistance against rapid RNAse digestion, enters the mesangium or the subepithelial space in mesangio- or membranoproliferative types of glomerulonephritis (33). In contrast to the low expres-
of TLR3 in unstimulated mesangial cells, activated mesangial cells express high levels of TLR3 and respond to dsRNA exposure. These mechanisms may contribute to the understanding of viral infection-associated glomerulonephritis.

Lupus Nephritis

Systemic lupus erythematosus is characterized by autoimmunity against various chromatin particles. Lupus nephritis develops when circulating chromatin-Ig-containing immune complexes are deposited in the kidney, followed by activation of the complement system and, subsequently, glomerular cells. A recent study from our laboratory provides evidence that circulating viral dsRNA can aggravate lupus nephritis in MRL<sup>lpr/lpr</sup> mice through the activation of mesangial cells and intrarenal macrophages (54). In fact, pI:C RNA induced mesangiolysis in nephritic MRL<sup>lpr/lpr</sup> mice. As pI:C RNA did not affect DNA autoantibody production and glomerular immune complex deposits, mesangiolysis was most likely caused by a direct effect of pI:C RNA on mesangial cells, e.g., by inducing mesangial cell apoptosis as observed in cultured human mesangial cells (80).

Two other studies from our laboratory demonstrate that different mechanisms are involved when MRL<sup>lpr/lpr</sup> mice with lupus nephritis are exposed to bacterial CpG-DNA or viral ssRNA. Similar to dsRNA, injected CpG-DNA and ssRNA bound to macrophages and dendritic cells in glomeruli and tubulointerstitial areas in nephritic kidneys (5, 56). Uptake into intrinsic renal cells was not detected, consistent with a lack of TLR7 and TLR9 immunostaining on intrinsic renal cells. Treatment with bacterial CpG-DNA or the TLR7 agonist imiquimod markedly induced local chemokine expression in immune cell infiltrates that stained positive for TLR7 and TLR9. This was associated with an increase in periglomerular and interstitial macrophage and T cell infiltrates. CpG-DNA and imiquimod severely aggravated glomerular and tubulointerstitial damage in MRL<sup>lpr/lpr</sup> mice. Furthermore, bacterial CpG-DNA markedly increased production of dsDNA autoantibodies and glomerular IgG deposits. This was less prominent in imiquimod-treated mice, consistent with the observation that ssRNA/TLR7 interaction cannot activate B cells in the absence of additional cofactors (10). However, these findings are in contrast to the lack of autoantibody induction in viral dsRNA-treated MRL<sup>lpr/lpr</sup> mice (54), which should relate to the lack of TLR3 expression on B cells.

Together, viral dsRNA can aggravate lupus nephritis locally through TLR3 on renal macrophages, dendritic cells, and glomerular mesangial cells. DsRNA-induced disease activity is independent of B cell activation and humoral anti-chromatin immunity in experimental SLE and therefore differs from effects of circulating TLR7 ligands and bacterial CpG-DNA. Apparently, immune modulation caused by microbial nucleic acids relates to the specific expression pattern of their respective pattern-recognition receptors, i.e., TLR3 for viral dsRNA, TLR7 for viral ssRNA, and TLR9 for bacterial (or viral) ssRNA Viruses | dsRNA Viruses | DNA Viruses

<table>
<thead>
<tr>
<th>ssRNA Viruses</th>
<th>dsRNA Viruses</th>
<th>DNA Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C virus</td>
<td>Reoviruses</td>
<td>Human herpes viruses:</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>Rotaviruses</td>
<td>HHV1/2 (herpes simplex virus)</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td></td>
<td>HHV3 (Varicella zoster virus)</td>
</tr>
<tr>
<td>Hantavirus</td>
<td></td>
<td>HHV4 (Epstein-Barr virus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HHV5 (cytomegalovirus)</td>
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<tr>
<td></td>
<td></td>
<td>Hepatitis B virus</td>
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<tr>
<td></td>
<td></td>
<td>Polyomaviruses:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BK virus, JC virus</td>
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<tr>
<td></td>
<td></td>
<td>Parvoviruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenoviruses</td>
</tr>
</tbody>
</table>

ss, Single-stranded; ds, double-stranded.

Fig. 2. Activation of renal cells by microbial nucleic acids. A: circulating microbial nucleic acids or chromatin-containing immune complexes are taken up by mesangial cells and renal dendritic cells or macrophages. Activation of the respective TLR leads to production of proinflammatory mediators and cell type-specific functions. GBM, glomerular basement membrane. B: on local chemokine production, leukocytes recruit to the glomerular and tubulointerstitial compartment and contribute to cytokine release and local inflammation.
CpG-DNA (Table 3). Next, the question arises as to which type of microbial nucleic acids has the greatest potential to induce lupus nephritis in a genetically predisposed host. We have addressed this question by injecting either pIC RNA, imiquimod, or CpG-DNA into young MRL wild-type and MRL$^{lpr/lpr}$ mice. Only CpG-DNA induced lupus nephritis in young MRL$^{lpr/lpr}$ mice, most likely due to its potential to activate B cells to produce autoantibodies and to secrete much higher levels of proinflammatory cytokines in immune cells as seen with TLR3 or TLR7 ligands (Pawar RD et al., unpublished observations).

**Renal Vasculitis**

The disease activity of renal vasculitis is considered to be associated with microbial infection (58). In fact, antibiotic treatment aiming to eradicate staphylococci from their internal reservoirs is under discussion for maintenance treatment of Wegener’s granulomatosis (67). The mechanisms that link microbes to disease activity of systemic vasculitis remain to be determined (58). In view of the data discussed above, microbial nucleic acids (as well as other microbial TLR ligands) may activate immunity in systemic and renal vasculitis. Furthermore, TLR3 is expressed on cultured human vascular endothelial cells (71). Immunostaining for TLR3 is also positive on vascular smooth muscle cells of arterioles and mid-sized arteries in the human kidney (80). These findings raise the interesting question of whether microbial nucleic acids contribute to the disease activity of vasculitis. However, the lack of appropriate animal models for renal vasculitis and the methodological problems in localizing microbial nucleic acids in human renal biopsies hamper an experimental approach to this hypothesis, at present.

**Transplant Rejection**

Infections are well-known triggers of renal allograft rejection (37, 64). Cytomegalovirus and polyomaviruses are DNA viruses that may activate TLR9 on immune cells that reside in the allograft and thereby contribute to renal inflammation and subsequent allograft dysfunction (69). Studies that address the role of TLR9 in this setting are not yet available. However, TLR signaling through MyD88 has shown to modulate autoimmunity. For example, MyD88-deficient mice did not develop minor antigen-mismatched allograft rejection of skin allografts (22). Fully major histocompatibility complex-mismatched skin or heart allograft rejection occurred independently of MyD88, although MyD88-deficient mice showed diminished dendritic cell-dependent T cell priming and Th1 responses (70). These data indicate that allograft rejection is mostly independent of MyD88, which is consistent with another study that did not find a contribution of TLR4 for solid organ transplantation recipients across major and minor histocompatibility barriers (60). Recent studies support a role for TLR4 and TLR2 in ischemia-reperfusion injury (72, 79), which would support the still hypothetical role of TLRs for recognizing endogenous “danger signals” in vivo (9, 49, 63). Together, TLRs may be involved in different aspects of renal transplantation, but experimental evidence is still limited to date.

**INTERFERING WITH TLR SIGNALING**

Specific antagonists for nucleic acid-specific TLRs are not yet available. However, the immunostimulatory effects of CpG-DNA can be neutralized with synthetic ODN of certain inhibitory sequence motifs (7). We injected such G-rich inhibitory ODN into MRL$^{lpr/lpr}$ mice from week 11 to 24 of age. Compared with saline-injected MRL$^{lpr/lpr}$ mice, inhibitory ODN prevented systemic lupus and lupus nephritis associated with decreased production of dsDNA autoantibodies and glomerular immune complex deposits (55). These data are consistent with a recent study that injected inhibitory ODN expressing TTAGGG motifs into the spontaneous lupus model of NZB/NZW mice (18). As in these studies, autoimmune mice had not been exposed to microbial DNA; these data argue for a role of endogenous CpG-DNA for dsDNA autoantibody production (59). This was confirmed by a recent study that characterized TLR9-deficient MRL$^{lpr/lpr}$ mice (14). Together, these data suggest that interfering with nucleic acid-specific TLRs may offer a new understanding of the pathogenesis of a number of kidney diseases and potentially new targets for therapeutic intervention (44).

**FUTURE DIRECTIONS**

Despite significant advances in the understanding of the TLR biology, many more questions toward an understanding of nucleic acid-related immune modulation remain. These include the following.

**Microbial Nucleic Acids in the Human Kidney**

At present, the relevance of these in vitro and experimental data for human kidney disease remains uncertain for two reasons. First, mostly synthetic mimics of microbial nucleic acids have been used in vitro and in vivo. Second, experimental infection models with renotrophic viruses need to be conducted in TLR-deficient mice to confirm the outlined hypotheses. Another approach would be the detection of microbial nucleic acids in human renal biopsies within the endosomal compartment of immune cells or mesangial cells. However, this approach is difficult, because the nature of the pathogen is usually unknown. However, HCV RNA was detected in immune complex precipitates in patients with HCV-associated cryoglobulinemic glomerulonephritis (32). HIV RNA was also detected in renal biopsies of patients with HIV-associated glomerulosclerosis (47, 78).

**Uptake Mechanisms**

Several routes for the uptake of microbial nucleic acids exist, but their molecular mechanisms remain poorly defined. Nucleic acids may enter the cell by phagocytosis of an entire

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**Table 3. Nucleic acid-specific modulation of lupus-like immune complex glomerulonephritis**

<table>
<thead>
<tr>
<th>Nucleic Acid</th>
<th>TLR</th>
<th>Cells</th>
<th>dsDNA Autoantibodies</th>
<th>IC Deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsRNA</td>
<td>TLR3</td>
<td>Mesangial cells, macrophages, dendritic cells</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ssRNA</td>
<td>TLR7</td>
<td>Macrophages, dendritic cells, B cells</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CpG-DNA</td>
<td>TLR9</td>
<td>Macrophages, dendritic cells, B cells</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

TLR, Toll-like receptor; IC, immune complex; –, response absent.
microorganism, apoptotic cell bodies, or just necrotic cell debris. Viral capsids may be taken up by membrane fusion, which may eventually fuse with intracellular endosomes and thereby deliver viral RNA to TLRs. However, RNA viruses that deposit their RNA into the cytosol are recognized by a different group of cytosolic RNA receptors, e.g., retinoic acid-inducible gene-I (83). The observation that cellular mRNAs or siRNAs can activate TLR3 raises the same question (12, 36). Cellular uptake of CpG-DNA is a saturable process (66), but what molecules mediate this process?

Intracellular Localization of TLR3 and TLR7/8

Whether TLR3 and TLR7/8 also reside in the ER before they redistribute to endosomes that contain nucleic acids is unknown to date.

Molecular Ligand-Receptor Interaction

What are the mechanisms that recognize nucleic acids in the early phagosome as a prerequisite for triggering the redistribution of the respective TLR from its intracellular reservoir?

Sequence-Specific Modulation of TLR9

Different classes of CpG-ODN have been shown to cause different immunostimulatory activities, e.g., the induction of type I interferons or dendritic cell maturation (75). What are the additional factors that allow sequence-specific recognition of the different classes of CpG-DNA and that modulate the MyD88-signaling pathway?

Specificity or Redundancy of Single TLR Pathways

In real life, the infection will expose the host rather to entire pathogens than just to single TLR ligands. Costimulation of nucleic acid-specific TLR was shown to have additive effects on cytokine production in dendritic cells (53). The contribution of single TLR ligands compared with exposure to the entire microorganism in vivo remains to be determined.

Role of TLRs on the Renal Vasculature

TLR3 activation on renal vascular endothelial or smooth muscle cells may play a role in infection-associated vascular injury, e.g., in thrombotic microangiopathy, renal vasculitis, or postinfectious endocapillary proliferative glomerulonephritis. The regulation and function of TLR3 on these cells types need to be explored to address this issue.

SUMMARY

Different types of microbial nucleic acids are recognized by specific Toll-like receptors, which create a subgroup among the Toll-like receptor family. The cell type-specific expression profiles of single TLRs cause immune responses specific for each type of nucleic acid (Table 3). The finding that mesangial cells express TLR3 and produce proinflammatory cytokines on exposure to dsRNA gives rise to the hypothesis that selected microbial nucleic acids are involved in the pathogenesis of certain forms of glomerulonephritis. Microbial nucleic acids do also activate immune cells that reside in the kidney to produce proinflammatory mediators. In addition, bacterial CpG-DNA and viral ssRNA activate B cells, which can affect kidney diseases that are associated with autoantibody production, e.g., lupus nephritis.

NOTE ADDED IN PROOF

Recently, a number of cytosolic receptors have been recognized which mediate TLR-independent recognition of microbial nucleic acids (reviewed in Ref. 1). Their expression and function during kidney disease are not known. Wu and Peng describe a different phenotype of TLR9-deficient MRL<sup>F</sup> <sup>nomp</sup> mice as previously reported in Ref. 14 (Wu X and Peng SL. Toll-like receptor 9 signaling protects against murine lupus. <i>Arthritis Rheum</i> 54: 336–342, 2006). Thus the role of TLR9 in lupus nephritis is under debate. TLR2 was shown to mediate renal ischemia-reperfusion injury, which argues for the contribution of endogenous ligands in TLR signal (Leemans JC, Stokman G, Claessen N, Roussch KM, Teske GJ, Kirschning CJ, Akira S, van der Poll T, Weening JJ, and Florquin S. Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. <i>J Clin Invest</i> 115: 2894–2903, 2005).

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REFERENCES


Invited Review

F516 NUCLEIC ACIDS AND TOLL


