Innate immunity, pattern recognition receptors, and inflammasomes in diabetes and diabetic nephropathy

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Abstract: The innate immune system consists of several classes of pattern recognition receptors (PRRs), including membrane-bound Toll-like receptors (TLRs) and nucleotide-binding domain leucine-rich oligomerization domain (NOD)-like receptors (NLRs). These receptors detect pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) in the extracellular and intracellular space. Intracellular NLRs constitute inflammasomes, which activate and release caspase-1, IL1β, and IL18 and thus initiate an inflammatory response. Systemic and local low-grade inflammation and release of proinflammatory cytokines are implicated in the development and progression of diabetes mellitus and diabetic nephropathy. TLR2, TLR4, and the NLRP3 inflammasome are critically involved in the inflammatory responses in pancreatic islets, adipose, liver and kidney tissues. This Review discusses how innate immune system-driven inflammatory processes can lead to apoptosis, tissue fibrosis, and organ dysfunction to cause insulin resistance, impaired insulin secretion, and renal failure. We propose that careful targeting of TLR2, TLR4, and NLRP3 signalling pathways may be beneficial for the treatment of diabetes mellitus and diabetic nephropathy.

Key points

- The innate immune system consists of several classes of pattern recognition receptors, including TLRs and NLRs, which detect pathogen-associated and danger-associated molecular patterns and initiate an inflammatory response
- TLR2 and TLR4 are the predominant toll-like receptors expressed on pancreatic β cells that trigger an inflammatory response in insulitis during type 1 diabetes mellitus
- TLR2 and TLR4 signaling, and activation of NLRP3 inflammasomes results in production of various proinflammatory cytokines that can induce insulin resistance in type 2 diabetes mellitus (T2DM) and obesity
- Innate immune responses in T2DM and obesity are modulated by the status of the gut microbiota, autophagy, and adipokines
- TLR2, TLR4, NOD2, and NLRP3 inflammasome-mediated inflammation are involved in the perpetuation of inflammation in diabetic nephropathy
- The activation of TLRs and NLRs stimulates the expression of MCP-1, which is associated with the progression of diabetic nephropathy

[H1] Introduction

The prevalence of diabetes mellitus is estimated to be 8.3%, affecting ~387 million people as of 2014. The number of patients living with diabetes mellitus is expected to increase to ~592 million by 2035, as reported by the International Diabetes Federation[1]. The incidence of end-stage renal disease (ESRD) is also rapidly increasing worldwide but varies up to 15-fold by country. In 2012, the number of new diagnoses of ESRD worldwide ranged from 25 to 467 patients per million. The proportion of new cases of ESRD with diabetes mellitus as the primary cause also differs by country, with 66% cases reported in Singapore and 12–16% cases reported in Ukraine, Romania, and the Netherlands[2]. Although intensified multifactorial intervention in patients with type 2 diabetes (T2DM) reduces the risk of

macroangiopathy and microangiopathy[3], 77% of affected patients live in low-income and middle-income countries where efficient therapeutic modalities for T2DM and its complications may not be fully available. Chronic inflammation is regarded as an important mechanism for the development of both diabetes mellitus and associated vascular diseases, such as diabetic nephropathy[4]. The adaptive immune response consists of humoral immunity mediated by antibody production by B cells, and cell-mediated immunity mediated by T cells. Although adaptive immune responses are important in the pathogenesis of type 1 diabetes (T1DM), the identification of new molecules associated with the innate immune system has prompted many researchers to investigate their role in the sustained inflammation in both T1DM and T2DM.

In contrast to adaptive immune system, the innate immune system can detect and destroy the majority of insults elicited by microorganisms within minutes or hours, using defense mechanisms that are independent of clonal expansion of antigen-specific lymphocytes[5]. These mechanisms include the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). In addition to sensing microorganism byproducts, the immune system has evolved to detect endogenous danger signals through the sensing of danger-associated molecular patterns (DAMPs)[6]. DAMPs are derived from damaged or dying cells, and include small molecules (ATP and DNA), particles (uric acid crystals and exogenous noxious factors such as asbestos) and environmental insults such as UV irradiation[7]. Systemic and local low-grade inflammation and release of proinflammatory cytokines are implicated in the pathogenesis of T1DM and T2DM. Furthermore, among the vascular complications of diabetes mellitus, subclinical inflammation is also known to contribute to the pathogenesis of diabetic nephropathy[4,8,9]. In this Review we provide an update on the functional roles of Toll-like receptors (TLRs) and NOD-like receptors (NLRs) in the pathogenesis of T1DM and T2DM, and its vascular complications, namely diabetic nephropathy[10]. We especially focus on the role of TLR2, TLR4 and NLRP3 and discuss the benefits and limitations of targeting these molecules in

the treatment of diabetes mellitus and diabetic nephropathy.

[H1] PRRs of the innate immune system

[H2] Toll-like receptors

The innate immune system consists of several classes of PRRs, including membrane-bound TLRs and C-type lectin receptors (CLRs), which detect PAMPs and DAMPs in the extracellular space and endosomal compartment. TLRs are type 1 membrane glycoproteins with a characteristic cytoplasmic Toll/interleukin-1 receptor (TIR) domain and a leucine-rich repeat domain[11,12]. TLRs recognize PAMPs, such as lipopolysaccharide (LPS), lipopeptides, flagellin, bacterial DNA, and viral double-stranded RNA (dsRNA)[13], as well as endogenous DAMPs, including HMGB1 and β-defensins[11]. The TLR signaling pathways are finely tuned by TIR domain-containing adaptors, such as MyD88, TIR domain-containing adaptor protein/MyD88 adaptor-like (TIRAP), TIR-domain-containing adaptor-inducing IFN-β (TRIF), and TRIF-related adaptor molecule (TRAM). The TIR domain consists of three boxes of conserved residues set in a core sequence of 135–160 amino acids, which facilitate receptor–adaptor oligomerization[14]. The differential recruitment of these adaptor proteins results in the formation of a myddosome complex—an oligomeric signaling complex that consists of the adaptor proteins MyD88, IRAK1 and IRAK4—and activation of various signaling molecules, such as NFκB, MAPK, and interferon regulatory factor (IRF) and the subsequent production and release of various cytokines and chemokines (Figure 1; Table 1)[15-19].

[H2] NOD-like receptors

Another set of intracellular sensing PRRs are NLRs, which are localized in the cytosol and are essential for sensing invading pathogens and prompting the innate immune response[20]. Intracellular NLRs organize signaling complexes such as NOD signalosomes (a protein complex involved in the regulation of protein degradation by ubiquitination)[21] and

inflammasomes (a multi-protein molecular scaffold complex)[22]. NOD1 and NOD2 are well-characterized NLRs in the NOD signalosome, which upon activation homo-oligomerize and recruit signaling molecules that drive NFκB-dependent and AP1-dependent production of proinflammatory cytokines[23]. Some inflammasomes have been shown to activate caspase-1, convert premature forms of IL1β, IL18, and IL33 to mature forms, and secrete these cytokines into the extracellular space[24]. Each NLR contains N-terminal effector domains, such as the caspase activation and recruitment domain (CARD), pyrin domain (PYD), and the baculoviral inhibitor of apoptosis protein repeat domain (BIR), and are divided into subfamilies based on the composition of these domains[25-27]. All NLRs contain a central nucleotide binding and oligomerization (NACHT) domain and most members have a variable number of ligand-sensing C-terminal leucine-rich repeat (LRR) domains[25-27].

[H2] Inflammasomes

Several inflammasomes with different sensor proteins, such as NLRP1, NLRP3, IPAF, NLRP6, RIG-1, AIM2 oligomerize to form multi-protein inflammasome complexes. NLRP1 or NLRP3 oligomerize via the NACHT domain (a 300–400 amino acid NTPase domain), which results in subsequent PYD clustering and the recruitment of PYCARD, which contains a caspase recruitment domain (ASC) to activate caspase-1. Activation of caspase-1 promotes the processing and secretion of IL1β, IL18, and IL33 (**Figure 2**)[7,19,28].

[H1] Type 1 diabetes mellitus (T1DM)

[H2] Involvement of TLRs

T1DM is a well-established type 1 T helper (T_H1) cell-mediated disease whereby pancreatic β cells are selectively injured and destroyed[29]. Many of the studies that have addressed the mechanisms of T1DM have mainly focused on the role of the adaptive immune system that is mediated by T lymphocytes. The discovery of TLRs has, however, led to a better understanding of the signaling pathways involved in the innate immune response, which has

an important role in promoting the autoreactive T-cell response that can initiate T1DM[30]. Experimental evidence to support the involvement of TLRs in T1DM is discussed below.

[H3] TLR2 and TLR4

A *TLR2* single nucleotide polymorphism haplotype, TLR2-Ht4, is strongly associated with T1DM; more than one copy of TLR2-Ht4 has been demonstrated to confer strong protection from the development of T1DM[31]. Peptide p277, a 24-amino acid fragment of HSP60, has also been shown to arrest the spontaneous diabetogenic process that occurs in non-obese diabetic (NOD) mice following therapeutic vaccination[32]. p277 induces a cytokine profile shift from T_H1 to type 2 T helper cells (T_H2) through the stimulation of TLR2 signaling[32]. This shift was shown to promote an up-regulation of integrin-mediated adhesion to fibronectin and inhibition of chemotaxis to SDF-1α *in vitro*[32]. Furthermore, the treatment of antigen-presenting cells in NOD mice with zymosan—a fungal cell wall component that interacts with TLR2 and dectin 1—resulted in increased suppressor function of $CD4^+CD25^+$ regulatory T (T_{REG}) cells, increased frequency of IL10, IL17, IL4, and Foxp3-positive T cells as determined by FACS analysis, suppression of insulitis, and a marked delay in hyperglycaemia[33,34]. In addition Pam3CSK4—a synthetic TLR2 agonist—prevented the development of T1DM in NOD mice by promoting the number and function of T_{REG} cells and conferring dendritic cells with tolerogenic properties[35,36]. Although TLR2 signaling through dendritic cells and T_{REG} cells ameliorates the progression of T1DM, TLR2 and TLR4 are the main receptors on pancreatic β cells for HMGB1 and can trigger an inflammatory response during the development of diabetes mellitus in NOD mice[37]. Finally, murine islets constitutively express TLR2 and TLR4 and the activation with LPS (lipopolysaccharides) and peptidoglycans has been demonstrated to induce primary graft failure with mononuclear cell infiltration[38]. Transplantation of *Tlr2*$^{-/-}$ and *Tlr4*$^{-/-}$ islets into wild-type mice reduced proinflammatory cytokine production and improved islet survival[38]. Taken together, inhibition of TLR2 and TLR4 signaling is beneficial to suppress sustained inflammation in pancreatic

islets; however, this approach may prompt the initiation of an adaptive immune response against autoantigens in pancreatic islets.

[H3] TLR2-mediated atherosclerosis

Endothelial inflammatory responses mediated by TLR2 and TLR4 are involved in the progression of atherosclerosis in diabetes mellitus and obesity[39]. In cultured coronary artery endothelial cells derived from the patients with T1DM, treatment with TLR2 agonist peptidoglycans and TLR4 agonist LPS enhanced the expression of NFκB, ICAM-1, IL6, and IL8 compared to control cells[40]. Blockade of TLR2 in intact aortas using an anti-TLR2 antibody attenuated an increase in vascular contraction in the streptozotocin (STZ)-diabetic rat, as assessed by wire myography. The activation of TLR2 in primary aortic vascular smooth muscle cell cultures triggered activation of RhoA, which was exacerbated in cells from STZ-diabetic rats[41]. Single nucleotide polymorphisms in *TLR2* (Arg753Gln and T–16934A) and *TLR4* (Asp299Gly and Thr399Ile) were not associated with carotid artery intima-media thickness at baseline in genome-wide association studies of diabetes mellitus over a 3-year follow-up period,[42] and the *TLR2* polymoprhisms Arg677Trp or Arg753Gln, *TLR4* polymorphisms Asp299Gly or Thr399Ile and *TLR9* polymoprhisms T–1237C were not suitable markers to predict susceptibility to T2DM or coronary artery disease in the Chinese population[43].

[H2] Involvement of NLRs

NFκB increases the transcription of pro-IL1β through the signaling pathways that are downstream of the activation of TLRs. proIL1β is subsequently processed and cleaved to IL1β by the NLRP3 inflammasome, which consists of NLRP3, ASC, and caspase-1[44]. TLRs and IL1β are upregulated in T1DM and IL1β is known to induce apoptosis of pancreatic β cells[45]. Two multicenter randomized clinical trials were conducted to examine the effects of IL1 antagonism on pancreatic β cells[46]. Canakinumab (an anti-IL1β antibody) and anakinra

(an anti-IL1R blocker) were safe but not effective as single immunomodulatory drugs, and thus combination therapy with agents that target the adaptive immune system in organ-specific autoimmune disease may be required. The *NLRP3* rs10754558 single nucleotide polymorphism lies in the 3'-untranslated region of the gene[47]. This polymorphism was found to be significantly associated with T1DM in patients of Brazilian origin (*P*=0.004) and exerted a protective effect on the natural history of the disease[47]. *Nlrp1b* was identified as a strong candidate gene for T1DM susceptibility in both NOD and NOR (insulitis resistant and diabetes free despite genetic identity with NOD mice at numerous chromosomal regions) mice. *Nlrp1b* belongs to the NLR gene family, which contributes to the assembly of the inflammasome, recruitment of caspase-1, and release of IL1β[48]. Investigations into the role of the inflammasome in the development of T1DM have so far been limited and more research is required to answer the question as to whether intervention on the innate immune system by targeting inflammasommes will exert beneficial effects in T1DM.

[H1] T2DM, insulin resistance, and obesity

Subclinical and chronic inflammation in obesity and metabolic syndrome can induce insulin resistance and T2DM, and T2DM associated with obesity is now considered as an inflammatory chronic disease[49]. The low-grade inflammatory state in adipose tissues, skeletal muscle, liver, gastrointestinal tract, and pancreas develops in response to an excess of nutrient flux, and the innate immune response that is mediated by TLRs and NLRs is an important link with insulin resistance [50]. Adipose tissue is an important site of inflammation and is host to various inflammatory cells, such as neutrophils, basophils, M1 and M2 macrophages, T_H1, T_H2, T_{REG} cells, $CD8^+$ cells, and B cells, and is an important source of various chemokines, cytokines, and adipokines that modulate inflammatory responses in obesity and T2DM[51].

[H2] Involvement of TLRs

[H3] TLR2

Mice lacking *Tlr2* are substantially protected from diet-induced adiposity, insulin resistance, hypercholesterolaemia, hepatic steatosis, and macrophage infiltration, and the level of inflammatory cytokines are reduced compared to wild-type mice [52,53]. Furthermore, the absence of TLR2 was shown to attenuate local inflammatory cytokine expression and enhance insulin action in the liver[54]. Although TLR2 is implicated in the inflammatory response in high-fat diet (HFD)-induced obesity in rodents and in human gene expression studies[55], saturated and polyunsaturated fatty acids did not elicit proinflammatory effects in human adipose tissue and primary adipocyte culture [56]. Conversely, administration of a HFD for 1 week to healthy males reduced the expression of *TLR2* [57]. Such discrepancies between animal and human investigations complicate the understanding of the molecular mechanisms underlying T2DM and obesity, and further clinical trials are required to confirm the benefits of inhibiting TLR2 signaling in patients.

[H3] TLR adaptor molecules

Intracellular adaptor molecules, such as MyD88, and signal transducers, such as IRAK and TRAF family members, can also act as ligands to TLRs and can activate NFκB and members of the interferon response family (IRF). In addition to the MyD88-dependent pathway, TLR4 recruits TRIF to activate downstream signaling cascades and the activation of IRF3 (Figure 1). *Myd88*$^{-/-}$ mice fed a HFD exhibited increased circulating levels of insulin, leptin, and cholesterol, which is associated with insulin and leptin resistance and development of severe T2DM [58]. *Myd88*$^{-/-}$ mice also had smaller islets and abnormal glucose tolerance after treatment with low-dose streptozotocin—a naturally occurring chemical that has toxic effects on insulin-producing β cells[59]. Although these reports suggest a protective role of MyD88 in glucose metabolism, both *Trif*$^{-/-}$ and *Myd88*$^{-/-}$ mice are protected against lard-induced white adipose tissue inflammation and impaired insulin sensitivity [60]. Furthermore, mice with central nervous system (CNS) specific deletion of

Myd88 were protected from HFD-induced weight gain and leptin resistance induced by acute intracerebroventricular injection of palmitate[61]. *Myd88*$^{-/-}$ RIP–B7.1 mice that express B7.1 (CD80) costimulatory molecules in pancreatic β cells were protected from diabetes mellitus following polyinosinic:polycytidylic (poly I:C) treatment—a synthetic double-stranded RNA and agonist of TLR3 [62]. In addition, inducible intestinal epithelial cell-specific deletion of *Myd88* in mice protected against the development of obesity, diabetes, and inflammation, which could be recapitulated following transplantation of the gut microbiota to germ-free recipient animals[63].

The conflicting results obtained from *Myd88*$^{-/-}$mice may be explained by the complex crosstalk between the microbiota and acute phase emergency granulopoiesis that can occur within minutes of infection[64]. Microbiota-driven myelopoiesis that occurs at concentrations of microbial antigens and PAMPs that are below the threshold required for an adaptive immune response is important for the surveillance of infection[64]. The crosstalk between the innate and adaptive immune response, where MyD88 relays the signals of TLR-induced IL1 production, has been shown to be dispensable for T_H1 responses in the absence of T_{REG} cells[65]. The innate immune system mediated by MyD88, however, is essential in protection against infections, and inhibition of MyD88 in the treatment of T2DM and obesity should be considered carefully.

[H2] Obesity-associated inflammation

Three apparently distinct mechanisms have been proposed as major triggers for sustained obesity-associated inflammation. These mechanisms include TLR4 activation, changes in the gut microbiota, and endoplasmic reticulum stress. Extensive crosstalk also occurs between these three mechanisms, which will be discussed in more detail below.

[H3] TLR4

Changes in the gut microbiota, reduced defense barriers in the intestine, and increased leakage of LPS and fatty acids can lead to the activation of TLR4 pathways and inflammation[66]. Lack of *Tlr4* in mice fed a HFD resulted in protection against obesity[67], insulin resistance,[68] and inflammation in adipose tissues[69,70], liver, skeletal muscle[71], and vasculature[72]. Specific deletion of *Tlr4* in haematopoietic cells ameliorated insulin resistance in hepatic and adipose tissue[73] and mice with a specific loss of *Tlr4* in hepatocytes exhibited improved glucose tolerance, enhanced insulin sensitivity, and ameliorated hepatic steatosis[74].

The agonists for TLR2 and TLR4, such as lipopolysaccharides (LPS) and saturated free fatty acids, are involved in manipulating the signaling events via NFκB-p65 nuclear translocation and proinflammatory cytokine production that have been shown to occur in adipose, liver, muscle and pancreatic β cells of animal models of insulin resistance and obesity[55,75]. The inflammatory pathway mediated by IL6 and TNF is mainly induced by the activation of TLR2 and TLR4, as demonstrated in adipose tissues from adolescents with metabolic syndrome[76]. Transwell co-culture of U937 mononuclear cells showed that the activation of TLR4 by LPS or palmitate induced the production of IL6 and activation of MMP-1, which may be involved in adipose tissue remodeling and obesity[77]. However, human SW872 preadipocyte cells actively secrete HMGB1, which boosts production of IL6 via RAGE and does not involve TLR2 and TRL4 signalling[78].

Evidence thus far suggests that free fatty acids do not directly bind to TLR4, and thus an endogenous ligand for TLR4 remains to be identified[79]. Fetuin-A has been reported as an endogenous ligand to TLR4 and knockdown experiments in mice with HFD-induced insulin resistance resulted in downregulation of TLR4-mediated inflammatory signaling in adipose tissue[79]. S100A8 and S100A9 are endogenous DAMPs that belong to the S100 calgranulin family. These DAMPs can induce insulin resistance and TLR4 and NLRP3

inflammasome-dependent IL1β production in adipose tissue, macrophages, and neutrophils in both mice and humans[80]. A small-molecule inhibitor of TLR4 signaling, TAK-242, provides complete protection against LPS-induced and non-esterified fatty acid-induced inflammation in muscle cells and partial protection against insulin resistance [81]. The identification of endogenous ligands and their inhibitors is required to facilitate the development of therapeutic agents to ameliorate inflammation, insulin resistance, and obesity.

[H3] Gut microbiota

Evidence that the composition of the gut microbiota differs between healthy individuals and obese and/or patients with T2DM has spurred investigations into the link between the pathophysiology of metabolic diseases and the gut microbiota. *Tlr2*$^{-/-}$ mice bred and maintained under germ-free conditions are protected from diet-induced insulin resistance[82]. Under non-germ-free conditions these mice are characterized with insulin resistance and obesity associated with changes in the gut microbiota, including a threefold increase in *Firmicutes* and a slight increase in *Bacteroidetedxvsafs*[82,83]. These data suggest that TLR2 has an important role in maintaining healthy microbiota and preventing obesity and insulin resistance. Similarly, *Tlr4*$^{-/-}$ mice fed a HFD demonstrated reduced intestinal permeability, increased plasma endotoxin and proinflammatory cytokine levels, and increased epididymal fat weight[84]. Interestingly, chronic intake of a HFD leads to severe pulmonary damage and mortality in *Tlr2*$^{-/-}$ *Tlr4*$^{-/-}$ mice[85], suggesting that diet and innate immune deficiency have a strong impact on the composition of the gut microbiota, which can lead to life-threatening conditions.

In addition to TLR2 and TLR4, bacterial flagellin-recognizing *Tlr5*$^{-/-}$ mice demonstrated hyperphasia and metabolic syndrome, which was transmissible to wild-type mice by gut microbiota transplantation[86]. Taken together, total loss of TLRs may induce harmful changes to the microbiota and can result in the deterioration of insulin resistance and induce

obesity in experimental mouse models.

In a human study, participants with high expression levels of TLR5-signalling pathway genes in adipose tissues tended to have obese phenotypes and a higher abundance of flagellated *Clostridium* cluster XIV and *Firmicutes*:*Bacteroides* ratio compared to participants with low TLR5 expression levels[87], suggesting that upregulation of TLRs enhances the inflammatory response and deteriorates insulin resistance and promotes obesity. The interaction between the microbiota and TLRs can result in whole-body inflammation and insulin resistance; however, total knockout of TLRs such as *Trl2* and *Tlr4* also impairs the innate immune system and results in persistent inflammation in mice[88]. Thus, therapeutic interventions against TLRs in obesity and T2DM require specific attention to the changes in the gut microbiota induced by a HFD, as the complete blockade of TLRs has harmful effects in patients who are obese.

[H3] ER stress

Substantial crosstalk also occurs between TLRs and endoplasmic reticulum stress. *Tlr4*$^{-/-}$ mice fed a HFD were protected from endoplasmic reticulum stress compared to wild-type mice, as assessed by monitoring the markers of endoplasmic reticulum stress BiP, C/EBP homologous protein (CHOP), spliced and un-spliced XBP1, and phospho-eIF2α[89]. The activation of the TLR4 pathway attenuates adaptive thermogenesis via endoplasmic reticulum stress in mice and it may explain the development of compromised adaptive thermogenesis in obese patients[90]. The mice fed a HFD were administered a chronic low dose of LPS before cold acclimation, which resulted in a reduced core body temperature and heat release, and elevated levels of endoplasmic reticulum stress and autophagy[90]. In addition to TLRs, endoplasmic reticulum stress is known to activate the NLRP3 inflammasome and induce apoptosis in pancreatic β cells[91,92]. This scenario has also been demonstrated in macrophages[93] and adipocytes[94], and endoplasmic reticulum stress is

sufficient for the production of IL1β via the activation of NFκB and the NLRP3 inflammasome.

[H2] Involvement of NLRs

[H3] NOD2

The NOD2 signalosome and the NLRP3 inflammasome are both involved in the pathogenesis of obesity and insulin resistance. Expression levels of *Nod2* in splenocytes were found to be higher in C57BL/6 mice fed a HFD compared to control mice[95]. Furthermore, NOD2 contributes to the induction of inflammation through the activation of NFkB[95] and an HFD also increases *Nod2* expression in hepatocytes and adipocytes. *Nod2*$^{-/-}$ mice show increased inflammation of adipose tissue and the liver and exacerbated insulin resistance under an HFD that is associated with bacterial translocation from the gut to adipose tissue and the liver[96].

[H3] NLRP3 inflammasome

Besides NOD2, the NLRP3 inflammasome has a major role in regulating the innate immune system through its interaction with thioredoxin-interacting protein (TXNIP). Both *Txnip* and *Nlrp3* deficiency in mice resulted in similar phenotypes, with impaired activation of the NLRP3 inflammasome and improved glucose tolerance and insulin sensitivity[97]. The NLRP3 inflammasome senses lipotoxicity-induced increases in intracellular ceramide associated with caspase-1 cleavage in ATM, and *Nlrp3*$^{-/-}$ mice are prevented from insulin resistance and inflammasome activation in liver and adipose tissues[98]. Saturated fatty acids, such as palmitate, but not unsaturated oleate, induces activation of the NLRP3-ASC inflammasome causing caspase-1, IL1β, and IL18 production and reduced insulin tolerance and sensitivity[99]. In pancreatic islet cells, islet amyloid polypeptide also triggers the NLRP3 inflammasome and generation of mature IL1β, which is reversed by treatment with glyburide[100]. The loss of ASC (*Pycard*), a critical adaptor required for the assembly of

NLRP3, substantially improved insulin action and secretion by ameliorating the production of pancreatic IL1β and β-cell death caused by a long term HFD in mice [101]. The NLRP3 inflammasome, TLR2, and TLR4 are triggers for islet inflammation in T2DM and the activation of macrophages in various tissues is also mediated by activation of the NLRP3 inflammasome[102]. Pancreatic β-cell failure in the Zucker diabetic fatty rat is associated with activation of NLRP3-ASC infammasome in M1 macrophages infiltrating into pancreatic islets[103]. Furthermore, adipose S100A8 and S100A9 induces ATM to provoke TLR4/MyD88 and NLRP3 inflammasome-dependent IL1β production and stimulates IL1 receptors on bone marrow myeloid progenitors to contribute to the prominent monocytosis and neutrophilia observed in obesity[80].

[H3] NLRP3 interactions with the gut microbiota

Although NLRP3 is actively involved in the development of insulin resistance and impairment of insulin secretion, the NLRP3 inflammasome also responds to changes in the gut microbiota as a result from differences in the diet[104]. NLRP6 and NLRP3 inflammasomes and the effector IL18 unexpectedly ameliorate metabolic syndrome and slow the progression of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis via modulation of the microbiota[105]. Changes in the configuration of the gut microbiota as a result of inflammasome deficiency can cause exacerbation of hepatic steatosis and inflammation by the influx of TLR4 and TLR9 agonists into the portal circulation [105]. The hierarchy and tissue distribution of NLRs, complicated interactions between TLRs and NLRs, and alterations in intestinal microbiota associated with multiple inflammasome deficiencies could account for these discrepancies and conflicting results. The beneficial and adverse effects of targeting the NLRP3 inflammasome should be considered in relation to changes in the gut microbiota.

[H3] NLRP3–IL1 pathway

The injection of LPS into db/db mice induced production of IL1β in macrophages, and evidence of innate-immunity associated illness was greater in db/db mice compared to db/+ mice [106]. These effects could be blocked by administration of an IL1R antagonist[106]. The activation of NLRP3 has an important role in the production of IL1 in T2DM and it may be an important target for disease treatment [107,108]. Various factors induce the NLRP3–IL1 pathway, including: islet amyloid polypeptide deposition in the pancreas[100]; cholesterol crystals in atherosclerotic lesions[109]; Kilham rat virus in the spleen and lymph nodes of a LEW1.WR1 model of T1DM[110]; endoplasmic reticulum stress-inducing chemicals and free fatty acid palmitate in macrophages[94]; FFAs in atherosclerosis lesions[109] and adipose tissues[111]; extracellular deposition of ATP and P2X4 receptors in kidney[112]; TLR2/6 and TRL4 ligands in pancreatic islets and macrophages[113]; and IL22 in $CD4^+$ T cells[114]. In turn, various agents may block and suppress the amplification loop of NLRP3–IL1, such as α1-antitrypsin[115], losartan (angiotensin receptor blockers)[116], procyanidin B2,[117] and FOXO1 inhibitor[118] as shown in macrophages.

[H3] Autophagy

Autophagy is one of the main catabolic pathways responsible for maintaining intracellular homeostasis by the formation of autophagosomes and interactions with the endosome–lysosome pathways. The reduced capacity of autophagy with aging generates an inflammatory condition via activation of the NLRP3 inflammasome and such disturbed interplay between autophagy and inflammaosomes is linked to the pathogenesis of T2DM, obesity, and atherosclerosis[119]. Systemic impairment of autophagy by haploinsufficiency of the essential autophagy gene (*Atg7*$^{+/-}$) in ob/ob mice aggravated insulin resistance with increased lipid contents and inflammatory changes[120]. Furthermore, mice with a *Lyz2*-Cre-mediated knockout of *Atg5* in macrophages fed a HFD and administered LPS demonstrated systemic and hepatic inflammation[121]. Defects in macrophage autophagy results in abnormal polarization, with increased M1 and decreased M2 macrophage

subtypes, and enhanced hepatic inflammation and liver injury in obesity[121].

[H3] Effect of adipokines on NLRP3 inflammasomes

Finally, various adipokines, such as adiponectin, leptin, FGF21, RBP-4, BMP-4 and BMP-7, vaspin, apelin, and progranulin produced by adipocytes have a marked impact on the regulation of the inflammatory response in obesity and T2DM[122]. Leptin is an adipocyte-secreted hormone that inhibits food intake and stimulates energy expenditure; however, most obese individuals have hyperleptinaemia and they demonstrate leptin resistance[123]. Hyperleptinaemia is associated with subclinical inflammation in obese subjects and leptin enhances the secretion of IL18 from human monocytes via activation of caspase-1 inflammasomes[124]. FGF19 and FGF21 belong to a subfamily of FGFs that function as hormones and act on overlapping sets of cell surface receptors composed of classic FGF receptors and the β–Klotho complex, which has been associated with the regulation of glucose and lipid metabolism during the periods of fluctuating energy availability[125]. Pharmacologic application of FGF19 and FGF21 cause weight loss and improve insulin sensitivity and lipid metabolism[125]. Recent studies have suggested a link between FGF21 signaling and inflammation[126], and FGF21 may alleviate T2DM-associated vascular complications by inhibiting NFkB–NLRP3 inflammasome-mediated inflammation[127].

[H3] Atherosclerosis and NLRs

Cholesterol crystals have been shown to cause inflammation, and ultimately atherosclerotic lesions through the activation of the NLRP3 inflammasome[128]. Preclinical investigation by aortic pouch biopsy from patients with T2DM and atherosclerosis and studies in a pig model of T2DM identified downregulation of SIRT1 and AMPK[129]. AMPK is known to phosphorylate SREBP at Ser-327, inhibit its activity, and repress SREBP-dependent lipogenesis[129]. Once the lipogenic program is initiated, NLRP3, ASC, and IL1β are activated and upregulated[129].

By comparing patients undergoing coronary artery bypass graft with patients without atherosclerosis donating a kidney grafts, aortic *NLRP3* expression was found to be elevated in those with hypertension or diabetes mellitus, and smokers and was strongly correlated with coronary severity scores[130].

[H1] Diabetic nephropathy

During acute kidney injury (AKI), tissue necrosis produces endogenous agonists to stimulate the PRRs and trigger the innate immune system[131]. However, a functional role of PRRs has been questionable in chronic renal injury. The release of endogenous molecules stimulates the canonical signaling pathways via TLRs and NLRs and converges on the activation of proinflammatory responses[131].

[H2] Involvement of TLRs

The regulation of TLR2 and TLR4 has been implicated in the pathogenesis of various kidney diseases, including urinary tract infection, AKI, kidney transplantation, and glomerulonephritis[8]. Emerging evidence has demonstrated the pivotal roles of TLR2 and TLR4 in the perpetuation of inflammation in diabetic nephropathy[132].

High glucose conditions induce the overexpression of TLRs, such as TLR2 and TLR4 in endothelial cells in adipose tissue[72,133], the retina[134], coronary arteries[40], and kidney tissues[135]. Endothelial cells exposed to fluctuating glucose concentrations induce an upregulation of *TLR4* and *TLR2* expression and this process has been associated with increased NFκB, IL8, MCP-1, ICAM-1, and VCAM-1 (Figure 3). STZ-induced diabetic mice with *Tlr2* or *Tlr4* deficiency demonstrated no increased expression of glomerular ICAM-1[136]. Anti-adhesion molecule therapies may be beneficial to prevent macrophage infiltration in pancreatic islets, adipose tissues, liver, retina, atherosclerotic lesions and kidney tissues and may have beneficial effects in patients with diabetes mellitus and diabetic vascular

complications. Although an anti-ICAM-1 antibody (enlimomab) was tested in clinical trials in the context of stroke[137] and prevention of acute rejection in renal transplant recipients[138], enlimomab did not exert any beneficial effects and there have been no reports in the context of diabetic vascular complications, such as diabetic nephropathy. Targeting the signaling pathways of TLR2 and TLR4, however, may be a suitable to attenuate vascular inflammation in diabetes mellitus and diabetic nephropathy. Currently, inhibitors to TLR2 and TLR4 are undergoing clinical trials in various models of inflammatory disease and in subjects with obesity and T2DM (ClinicalTrials.gov Identifier NCT02267317)[139], although none in patients with diabetic nephropathy[132]. The specific involvement of TLR2 and TLR4 in diabetic nephropathy is discussed in more detail below.

[H3] TLR2

TLR2 and MyD88-mediated signaling were increased in macrophages obtained from STZ-mice, which was blunted following the complete ablation of *Tlr2*[140]. The characteristic features of diabetic nephropathy, such as renal hypertrophy, albuminuria, and podocyte loss were ameliorated in *Tlr2*$^{-/-}$-deficient STZ-induced diabetic mice[140]. Peritoneal and kidney macrophages were predominantly M1 phenotype in wild-type mice, and phenotypic changes of macrophages, podocyte loss and urinary albumin excretion were attenuated in *Tlr2*$^{-/-}$-deficient mice[140]. In STZ-induced and HFD-induced diabetic mice, TLR2 localized on glomerular endothelial cells and repeated *Porphyromonas gingivalis* LPS administration enhanced urinary albumin excretion and production of IL6, TNF and LTA (TNFβ) in glomeruli[141]. In human proximal tubular cells, HMGB1-induced NFκB activation was prevented by TLR2 silencing, and the researchers of this study emphasized the importance of TLR2 as a mediator of NFκB activation in diabetic nephropathy[142].

[H3] TLR4

In cultured mouse mesangial cells, high glucose (25 mM) increased *Tlr4* mRNA but not *Tlr2*

mRNA compared to low glucose (5.5 mM). The downstream signaling molecules, such as MyD88, IRF3, and TRAM were also markedly increased[143]. In patients with T2DM, fluorescent intensity of $CD14^+CD16^+$ cells, TLR4 expression, and serum levels of IL6 and CRP were higher compared to healthy control subjects[144]. Moreover, these biomarkers were further increased in uraemic patients with diabetic nephropathy, compared to patients with T2DM and healthy controls [144].

Blockade of TLR4 signaling by GIT27—an immunomodulatory agent that targets macrophages through the inhibition of TLR4 and TLR2/6-mediated signaling pathways—in *db/db* mice resulted in improved glycaemic control, decreased urinary albumin excretion, and improved glumeruloscleosis[145]. Another TLR4 antagonist, CRX–526, significantly reduced albuminuria and blood urea nitrogen without altering blood glucose and systolic blood pressure in eNOS knockout mice[146]. Compared with *Tlr4*$^{-/-}$ mice treated with STZ, wild-type mice treated with STZ demonstrated increased markers of fibrosis, such as type IV collagen and LTA (TGFβ) that was associated with increased macrophages, *TLR4* expression, MyD88, IRF3, NFκB activity, TNF, IL6, and MCP-1 in kidney tissues[146]. Furthermore, podocyte number and the expression of podocin was decreased in wild-type mice treated with STZ, which was reversed in *Tlr4*$^{-/-}$ mice[147]. This effect may be mediated by the prevention of podocyte injury and apoptosis by TLR4 inhibition[148,149].

Human studies have confirmed findings that glomerular expression of TLR4 is associated with an upregulation of *TNF*, *IL6*, *CCR2*, *CCL5*, and *CCR5* mRNAs and subsequent loss of renal function[150]. Taken together, TLR4 is a promising target for the treatment of diabetic nephropathy to suppress the inflammation process in the glomeruli (Figure 3). Elevated levels of TNF and IL6 have been detected in the sera of patients with diabetic nephropathy, which was positively correlated with elevated levels of human β-defensin, which stimulates TLR4 in dendritic cells[151]. Although therapeutic interventions for TNF and IL6 by use of

receptor fusion proteins and monoclonal antibodies were once regarded as an efficacious treatment for insulin resistance and diabetic nephropathy[152,153], there are no clinical data to prove the efficacy of inhibition of TNF and IL6 reported in the literature.

[H2] Involvement of NLRs

[H3] NOD2

Various studies support a role of NOD2 in the pathogenesis of diabetic nephropathy[154]. NOD2 was upregulated in HFD-induced and STZ-induced diabetic mice and kidney biopsies from patients with T2DM[154]. Furthermore, NOD2 was upregulated in podocytes treated with high glucose, AGEs, TNF, or LTA (TGFβ), and *Nod2*$^{-/-}$ mice were protected from hyperglycaemia-induced nephrin expression[154]. Thus, NOD2 links renal injury to inflammation and podocyte insulin resistance in diabetic nephropathy[154].

RNA-binding protein human antigen (HuR) is upregulated in patients with diabetic nephropathy, and correlates with the degree of proteinuria[155]. *In vitro* experiments using rat glomerular mesangial cells have indicated that high glucose can induce a prominent increase in cytoplasmic HuR, which allows HuR to bind to the 3'-untranslated region of *NOD2* and enhance the expression of *NOD2* and mRNA stability[155]. NOD1 and NOD2 were initially implicated in the pathogenesis of AKI by canonical signaling events, where tissue necrosis produces endogenous agonists to stimulate NODs and converge into the activation of proinflammatory responses[131]. NOD2 also elicits non-canonical signaling events in diabetic nephropathy and additional effects on LTA signaling in tubular cells, glucose handling, nephrin expression in podocytes, and macrophage phenotypes[131].

[H3] NLRP3

In addition to NOD2, NLRP3 inflammasome-mediated inflammation is recognized in the development of kidney injury, and urate and lipids are generally considered danger signals

in NLRP3 inflammasome activation. In an STZ-induced rat model of diabetic nephropathy with hyperuricaemia and dyslipidaemia, overexpression of NLRP3 inflammasome components (ASC and caspase-1) was associated with elevated IL1β and IL18[156]. Treatment with quercetin (a flavonoid) and allopurinol (a xanthine oxidase inhibitor) suppressed renal NLRP3 inflammasome activation and ameliorated diabetes-associated nephrotoxicity via anti-hyperuricaemic and anti-dyslipidaemic mechanisms[156,157].

Extracellular ATP is another endogenous signal that activates the NLRP3 inflammasome via stimulation of P2X receptors, which results in the production of IL1β and IL18[112]. Apyrase consumes extracellular ATP and can block the ATP-P2X4 signaling caused by high glucose, while the P2 receptor antagonist suramin, P2X receptor antagonist TNP-ATP, P2X4 selective antagonist 5-BDBD, and *P2x4* gene silencing attenuated NLRP3 expression and ameliorated renal NLRP3 inflammasome activation[112].

Mitochondria-derived reactive oxygen species (ROS) induced by high glucose conditions also stimulates the NLRP3 inflammasome, and abolishing NLRP3 or caspase-1 expression in bone marrow-derived cells fails to protect mice against diabetic nephropathy. Mice with specific non-myeloid derived cell *Nlrp3* deficiency, however, were protected against diabetic nephropathy despite transplantation of wild-type bone marrow[158].

In diabetic nephropathy, hyperuricaemia is a strong inducer of NLRP3 and IL1 production, which contributes to the progression of disease[158], and uric acid-lowering agents may demonstrate the therapeutic potential against diabetic nephropathy[156,157]. Hyperglycaemia induced the expression of thioredoxin-interacting protein (TXNIP), an inhibitor of thioredoxin, which activates the gp91 (phox), a subunit of NADPH oxidase, and NLRP3[159]. The inhibition of NADPH oxidase or TXNIP in STZ-induced diabetic C57BL/6 mice resulted in inhibition of the NLRP3 inflammasome and production of IL1. Overall, NADPH and/or TXNIP may be

suitable targets for the treatment of diabetic nephropathy[159].

Taken together, targeting the inflammasome may be a potential therapeutic approach for diabetic nephropathy; however, whether NLRP3 is the best molecular target or whether upstream molecules, such as NADPH oxidase, TXNIP, P2XR or NOD2 are more appropriate targets remains to be elucidated (Figure 3).

[H2] Influence of chemokines

The activation of TLRs and/or NOD1 stimulates the expression of chemokines, such as MCP-1 (CCL2) in adipose tissues in obesity[160-163] and in kidney tissue in hyperglycaemia. In particular, the expression of TLR2 and TLR4 was enhanced in STZ-induced rat models of diabetes mellitus [164,165] and global deletion of *Tlr4* in mice resulted in decreased renal inflammation and fibrosis associated with the reduction in the expression of MCP-1[147]. MCP-1 is a chemokine that binds to the CCR2 receptor that is expressed in monocytes and macrophages[166]. The activation of MCP-1 is driven by hyperglycaemia and glomerular hemodynamic perturbations in diabetes mellitus, and is an important target in the progression of diabetic nephropathy[167]. Some investigations have suggested that MCP-1 favors a shift from M1 macrophages to M2 macrophages in human macrophages[168,169], and macrophage polarization has been shown to shift from an M1 phenotype in the early phase of diabetes mellitus to an M2 phenotype in the later phases of the disease in an experimental animal model[170]. Thus, the inhibition of MCP-1 signaling seems to be beneficial in the treatment of diabetic nephropathy[170]. The CCR2 antagonist CCX140-B, and MCP-1 inhibitor, NOX-E36, have been developed and are currently under investigation in clinical trials.[170] The inhibition of MCP-1 may exert reno-protective effects in combination with renin-angiotensin-aldosterone system (RAAS) inhibition. CCR2 inhibition with CCX140-B exerts a renoprotective effect with capacity of lowering albuminuria on top of current standard of care with RAAS inhibition in patients with T2DM and diabetic nephropathy[171].

[H1] Conclusions

Sustained inflammation in pancreatic islets during T1DM is mediated by TLR2 and TLR4. Under excessive nutrition and obese states, TLR4 mediates inflammation both in pancreatic islets and in kidney tissues. In T2DM, TLRs sense hyperglycaemia and potential metabolic harm and translates this information by eliciting chronic inflammation. Thus, inhibition of TLR2 and TLR4 signaling may be beneficial to suppress inflammation and protect pancreatic β cells and kidney tissues from a decline in insulin secretion and renal function in diabetic nephropathy.

The interaction between the microbiota and TLRs can provoke whole-body inflammation and insulin resistance. Interestingly, total loss of TLRs can also impair the innate immune system and enhance inflammation. An active immune system has the potential to prevent the development and recurrence of cancer by immunosurveillance, but the tumor microenvironment can cause profound immune suppression and exhaustion[172]. TLR agonists activate both the innate and adaptive immune system and exert antiviral and antitumor activities. Inhibition of TLRs may accelerate the development of *de novo* tumorigenesis and recurrence of malignancies in patients with diabetes and diabetic nephropathy, and the utility of TLR inhibitors must, therefore, be considered with caution.

In the development of obesity and T2DM, elevation of IL1β is tightly associated with the development of insulin resistance and kidney injury. Activation of the NLRP3 inflammasome in adipose tissues, liver, and pancreatic islets is responsible for the development of obesity and T2DM. In diabetic nephropathy, the activation of NLRP3 inflammasome and production of IL1β is also responsible for the development of diabetic nephropathy. Thus, targeting the NLRP3 inflammasome may be a potential therapeutic approach for both diabetes mellitus and diabetic nephropathy.

Review criteria

PubMed was searched for peer-reviewed articles on May 7, 2015 with the following selection criteria: “TLR”, “NLR”, “NLRP3”, “inflammasome” or “innate immunity” in combination with “diabetes”, “diabetic nephropathy”, “insulin resistance”, “metabolic syndrome” and “obesity”. Only full-text papers published in English were considered. The initial literature screening of 2,378 articles was based on abstracts and selected full-text manuscripts were assessed before inclusion in this review article.

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Figure 1 Toll-like receptors (TLRs) and signaling molecules. The majority of TLRs seem to function as a homodimer, but TLR2 forms a heterodimer with TLR1, TLR6 or TLR10. TLR4 requires CD14 and MD2 for the recognition of lipopolysaccharides (LPS). TLR signaling consists of two distinct pathways; the TIRAP-MyD88-dependent and TRAM-TRIF-dependent cascades. TIRAP conducts the signal from TLR4 to MyD88 and TRAM mediates the signal from TLR4 to TRIF. The engagement of TLR7 and TLR8 on the plasma membrane and TLR9 in endosomes leads to the formation of a myddosome that consists of MyD88, IRAK1, and IRAK4. MAL binds to specific lipid microdomains to stabilize MyD88 interactions and trigger the formation of the myddosome. The activation of IRAK1 induces TRAF6 activation followed by activation of TAK1, MAPKs, AP1, and NFκB. TRAM is required for the activation of TRIF, which interacts with TRAF3, TRAF6, and RIPK1. TLR3 in the endosome recruits the TIR adaptor, TRIF, and mediates the TRIF-dependent pathway. TLR4 can also activate the TRAM-TRIF pathway following its CD14-dependent endocytosis and associated with endosomal-bound TRAM.

Figure 2 Nucleotide-binding domain leucine-rich repeat containing receptors (NLRs). Various DAMPs and PAMPs activate the formation of inflammasomes and several inflammasomes have been described which contain different sensor proteins such as NLRP1, NLRP3, IPAF, NLRP6, RIG-I and AIM2. The majority of inflammasomes require ASC to recruit caspase-1 and promote maturation and secretion of proinflammatory IL1β. The activation of TLR4 by LPS induces the activation of NFκB and enhances the transcriptional activities of NLRP3 and pro-IL1β. DAMPs, such as high fat diet causes reactive oxygen species (ROS) production from the mitochondria and DAMPs such as nanoparticles, CPPD, silica, asbestos, β-amyloid, and cholesterol crystals also causes the lysosomal rupture and release of cathepsins, which all activate the NLRP3 inflammasome. PAMPs, such as pore forming toxins, induce potassium efflux and it triggers the activation of

NLRP1 and NLRP3.

Figure 3 Innate immunity and inflammatory pathways in diabetic nephropathy and obesity-related kidney diseases. **A. Podocytes.** Thioredoxin (TRX)-interacting protein (TXNIP), a pro-oxidative stress and pro-inflammatory factor, activates the NLRP3 inflammasome by interacting with NALP3 in podocytes exposed to high glucose[173]. Under a high fat diet, mice lacking *P2x7r* exhibit reduced NLRP3, ASC, mature caspase-1, IL1β, and IL18[174]. In cultured podocytes, inhibition of NADPH oxidase abrogates NALP3 inflammasome activation and IL1β production, and eventually protects podocytes from high glucose-induced injury[159]. Mitochondrial reactive oxygen species (ROS) activate NLRP3 inflammasomes under conditions of high glucose or advanced glycation end-product stressed podocytes[158]. ASC is a component of NLRP3 inflammasome and shRNA-transfection of *Asc* in mice on a high fat diet attenuates proteinuria, albuminuria, foot process effacement of podocytes and loss of podocyte slit diaphragm molecules[175]. Human podocytes cultured with sera from normoalbuminuric patients with T1DM and high LPS activity show downregulation of 3-phosphoinositide-dependent kinase-1 (PDK1), an activator of the Akt cell survival pathway, and induction of apoptosis[148]. Both TLR2 and TLR4 knockout mice were protected from development of diabetic nephropathy[140,147,149]. B. Glomerular endothelial cells. In glomerular endothelial cells, mitochondrial ROS induced by high glucose and advanced glycation end-products (AGEs) provokes NLRP3 activation and production of caspase-1 and IL1β[158]. TLR2 and TLR4 also mediate the activation of NFκB and upregulation of MCP-1, IL8, ICAM-1 and VCAM-1, which result in the promotion of inflammation[136,141,176]. C. Mesangial cells. Although high glucose conditions increase the expression of TLR4, MyD88, IRF3, TRAM, activates NFκB, and enhances the expression of MCP-1 and IL6, functional studies of innate immunity in mesangial cells are lacking.

Table 1: Localization of toll-like receptors and ligands.

TLRs	Localization	PAMPs	DAMPs	Synthetic ligands
TLR-1 and -2	Cell surface	Triacylated lipoprteins Peptidoglycans Lipopolysaccharides (LPS)	Heat shock proteins High mobility groups proteins (HMGB1) Proteoglycans	Pam3CSK4
TLR-2 and -6	Cell surface	Diacylated lipoproteins	Heat shock proteins High mobility groups proteins (HMGB1) Proteoglycans	Pam2CSK4
TLR-3	Endosome	Viral dsRNA	mRNA tRNA	Poly(I:C), Poly(A:U)
TLR-4	Cell surface /endosomes	LPS	Heat shock proteins HMGB1 Proteoglycans Fibronectin Tenascin Amyloid β	Lipid A derivatives
TLR-5	Cell surface	Flagellin	Unknown	Recombinant flagellin
TLR-7	Endosome	Viral and bacterial ssRNA	Immune complexes Self RNA	Thiazoquinoline and imidazoquinoline compounds (R848)
TLR-8	Endosome	Viral and bacterial ssRNA	Immune complexes Self RNA	Thiazoquinoline and imidazoquinoline compounds (R848)
TLR-9	Endosome	Viral and bacterial CpG DNA	Chromatin Immune complexes Self RNA	Class A, B and C CpG oligodeoxynucleotides
TLR-10	Endosome	Unknown	Unknown	Unknown