Journal of Molecular Medicine (2018) 96:741–751
https://doi.org/10.1007/s00109-018-1660-7

JMolMed

REVIEW

# Epigenetic regulation of Toll-like receptors and its roles in type 1 diabetes

Zhiguo Xie [1,2] · Gan Huang [1,2] · Zhen Wang [1,2] · Shuoming Luo [1,2] · Peilin Zheng [1,2] · Zhiguang Zhou [1,2]

Received: 13 February 2018 / Revised: 8 June 2018 / Accepted: 11 June 2018 / Published online: 12 July 2018


## Abstract

The immune system can be divided into adaptive immunity and innate immunity. Adaptive immunity has been confirmed to be involved in the pathogenesis of autoimmune diseases, including type 1 diabetes (T1D). However, the role of innate immunity in T1D has only been studied recently. T1D is caused by selective autoimmune destruction of pancreatic islet $\beta$ cells. A series of studies have suggested that TLRs play a critical role in the pathogenesis of T1D. Aberrant TLR signaling will change immune homeostasis and result in immunopathological conditions such as endotoxin shock and autoimmune responses. Thus, TLR signaling pathways are supposed to be strictly and finely regulated. Epigenetics has recently been proven to be a new regulator of TLR expression. DNA methylation, histone modification, and microRNAs are the three main epigenetic modifications. This review will mainly focus on these epigenetic mechanisms of regulation of TLRs and the role of TLRs in the pathogenesis of T1D.

**Keywords** Toll-like receptor · Type 1 diabetes · Epigenetics · DNA methylation · MicroRNA

## Introduction

The immune system includes both innate immunity and adaptive immunity mechanisms, and their role is to identify and eliminate invading microbial pathogens [1, 2]. TLR signaling pathways are important for host defense, and aberrant TLR signaling will change immune homeostasis and result in immunopathological conditions such as endotoxin shock and autoimmune responses [3]. Therefore, TLR signaling pathways are supposed to be strictly and finely regulated [4, 5]. Type 1 diabetes (T1D) is generally considered to be caused by the interaction of the immune system with genetic susceptibility and environmental factors, such as viral infections [6–8]. Adaptive immunity has been confirmed to be involved in the pathogenesis of T1D. However, the role of innate immunity in T1D has only been investigated recently.

Epigenetic mechanisms can influence gene expression without changing the DNA sequence. DNA methylation, histone modification, and microRNAs are the three main epigenetic modifications. All of them are associated with transcriptional regulation and determination of the cellular transcriptome, thereby contributing to maintain normal cell function. Increasing evidence has shown that epigenetic mechanisms are involved in the regulation of TLRs. Since TLRs are involved in self vs. non-self-identification, they are thought to play an important role in the pathogenesis of immune disorders and autoimmune diseases [9]. Understanding the epigenetic mechanisms that regulate TLRs is thus important for the study of disease pathogenesis and the treatment of autoimmune diseases including T1D.

## Toll-like receptors (TLRs) and TLR signaling pathways

Toll was first identified to play an important role in recognizing microorganisms and establishing the dorsal-ventral axis during the embryogenesis of *Drosophila melanogaster* in 1996 [10]. The homolog of Toll in humans is called the Toll-like receptor (TLR). There are 10 functional TLRs (TLR1–TLR10) that have been identified in humans. Since their discovery, TLRs have gained increasing attention for

Zhiguo Xie and Gan Huang contributed equally to this work.

✉ Zhiguang Zhou
zhouzhiguang@csu.edu.cn

[1] Department of Metabolism & Endocrinology, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China

[2] Key Laboratory of Diabetes Immunology (Central South University), Ministry of Education, National Clinical Research Center for Metabolic Diseases, Changsha 410011, Hunan, China

their role in the innate immune system as well as for their involvement in cross talk between the innate and adaptive systems (Fig. 1).

TLRs can be divided into five subfamilies based on their sequence, genomic structure, and function [11]. Although all TLRs are membrane proteins, TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are localized on the plasma membrane. In contrast, TLR7, TLR8, and TLR9 are localized to intracellular vesicles, such as endosomes, lysosomes, and the ER, while TLR3 can be localized on both cell surfaces and endosomes [12]. TLRs are expressed in both innate and adaptive immune cells, such as dendritic cells, macrophages, neutrophils, B and T cells [13, 14], T regulatory cells [15], and on non-immune cells such as fibroblasts, epithelial cells, and islet beta cells [16]. However, the highest expression of TLRs is on tissues that are involved in innate immunity [17].

Different TLRs can recognize different pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) (Table 1). PAMPs are usually the pathogenic molecules produced by bacteria, fungi, parasites, and viruses, which can interact with cells of the innate immune system [1, 18]. DAMPs are the endogenous molecules released by necrotic cells as well as intracellular proteins, such as heat shock proteins [19]. TLRs are type 1 transmembrane glycoproteins that consist of an extracellular part of TLRs, with a leucine-rich repeat domain (LRR) that specifically recognizes PAMPs, and the intracellular domain of TLRs with a Toll/interleukin-1 receptor (TIR) domain that recruits downstream adaptor proteins [20, 21]. The activation of TLR signaling transduction pathways will result in a series of downstream signaling events that lead to the activation of transcription factors and ultimately cause the upregulation of various pro-inflammatory cytokines, chemokines, and interferons (IFNs) and activate the adaptive immune system [22, 23].

There are two TLR-mediated signaling pathways after TIR adaptor binding, namely the myeloid differentiation primary response 88 (MyD88)-dependent pathway and the TIR domain-containing adaptor-inducing IFN-β (TRIF)-dependent pathway [24]. The MyD88-dependent pathway is common to most TLRs. Upon ligand recognition, TLRs, except for TLR3, will recruit adaptor protein MyD88 and launch the pathway. This will lead to the expression of inflammatory cytokines. In contrast, TLR3 does not use the MyD88-dependent pathway but instead uses the TRIF-dependent pathway [25]. The TRIF-dependent pathway will cause activation of TRAF3 and subsequent activation of interferon regulatory factor 3 (IRF3) and mediates type I interferon (such as IFN-β) expression [26].

Generally, the activation of TLR signaling pathways will cause subsequent immune responses to eliminate pathogens. However, aberrant activation of TLR signaling pathways may disturb immune homeostasis. Thus, its aberrant activation can be involved in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus, multiple sclerosis, T1D, and cancer [27]. Given the importance of TLRs in host defense and the pathogenesis of disease, there must be a complex regulation mechanism to strictly modulate TLR signaling pathways.

## Epigenetic regulation of TLRs

Various mechanisms have been proposed to maintain the immunological balance that evolved to regulate TLR signaling pathways [4, 9, 28]. Epigenetics has recently emerged as a new method involved in the regulation of TLRs and has received increasing attention in the field. There are three main epigenetic modifications that take part in the regulation of TLRs: DNA methylation, histone modification, and non-coding RNA. We will discuss here the direct role of epigenetic modification in regulating TLRs (Fig. 2).

### DNA methylation and histone modification in TLR regulation

DNA methylation usually refers to the biochemical process in which a methyl group is added to the cytosine DNA nucleotides. DNA methylation is an important epigenetic control mechanism associated with gene regulation. Histones are the primary protein components of eukaryotic chromatin found in cell nuclei and play a role in gene regulation. H1, H2A, H2B, H3, and H4 are the major families of histones. The possible modification types of histones include acetylation, phosphorylation, methylation, ubiquitination, SUMOylation, and so on [29]. These modifications will alter the histone's interactions with DNA and lead to epigenetic changes, which may regulate disease processes .

Recently, the role of DNA methylation and histone modification in TLR regulation has been studied (Table 2). For example, Haehnel et al. [5] analyzed the expression of TLR2 in human monocytes and macrophages. They found that DNA methylation of the proximal human TLR2 promoter is associated with TLR2 repression. Zampetaki et al. [35] reported that TLR4 expression underwent DNA methylation and histone modification in mouse embryonic stem cells but not in embryonic stem cell-derived differentiated smooth muscle cells. Takahashi et al. [36] suggested that TLR4 gene transcription is downregulated by epigenetic modification, including histone deacetylation and DNA methylation, in human intestinal epithelial cells.

The above studies have provided us with evidence that the expression of TLRs can be directly regulated by DNA methylation and histone modification. We note that the studies regarding DNA methylation and histone modification in the direct regulation of TLRs are still limited. However, considering the important role of DNA methylation and histone

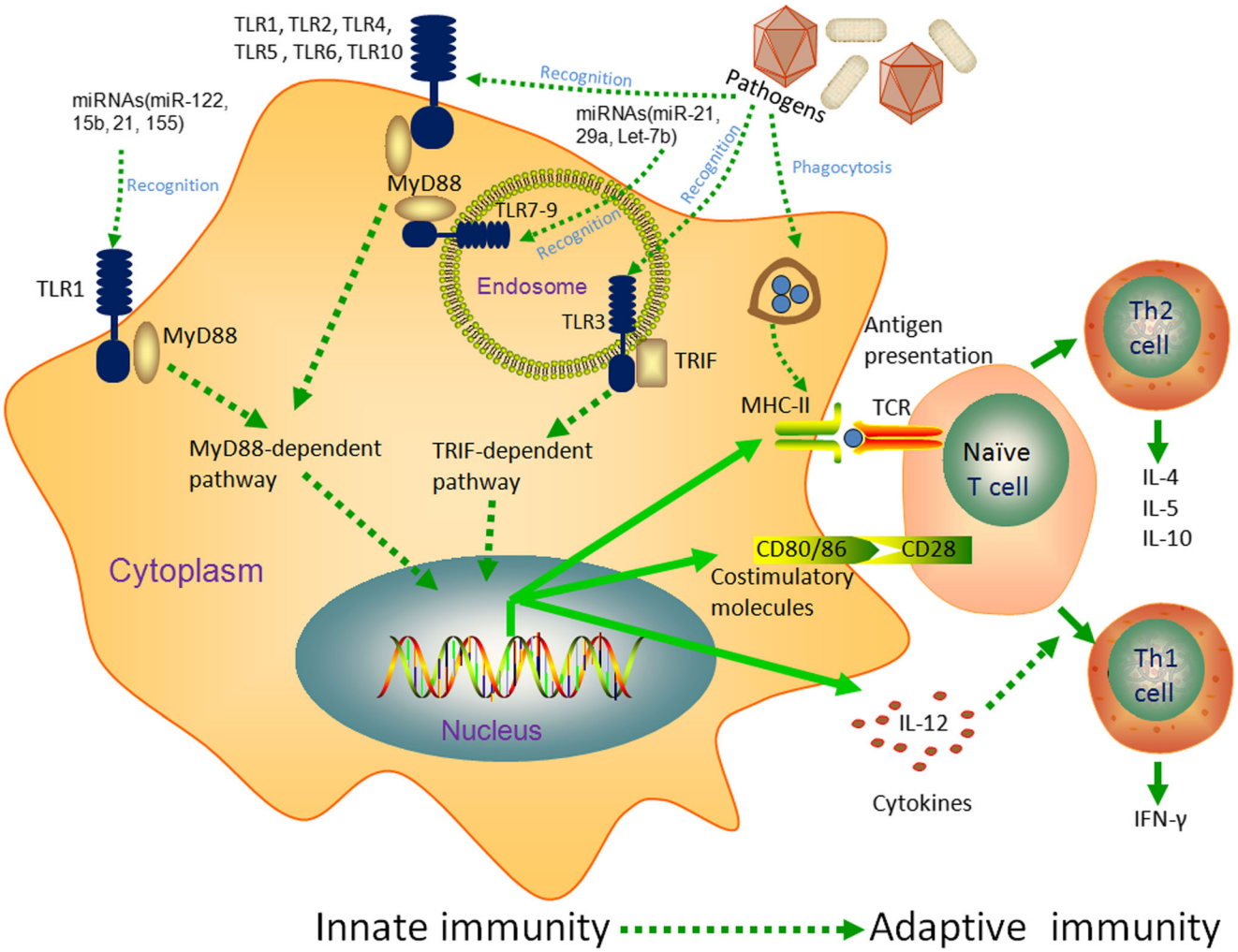


**Fig. 1** TLRs in innate and adaptive immunity. Pathogens, as well as some microRNAs, can be recognized by TLRs, and TLR signaling pathways will then be initiated, in turn upregulating the expression of co-stimulatory molecules (CD80/86), MHC-II, and inflammatory cytokines (such as IL-12). Recent studies have suggested that microRNAs serve as ligands of TLRs, such as TLR1, 7, and 8, which are shown in the figure. In addition, pathogens can be captured by dendritic cells and be processed and presented to naïve T cells. Together, these signals contribute to the development of antigen-specific adaptive immunity

modification in the regulation of gene expression, we believe that there will be increasing research on the regulation of TLRs by DNA methylation and histone modification.

## MicroRNAs and TLR signaling pathways

Non-coding RNAs (ncRNAs) are RNA molecules that are transcribed from DNA but have low protein coding potential [39]. MicroRNAs are the most studied ncRNAs to date. MicroRNAs usually induce gene silencing by binding to the 3′UTR of targeted mRNAs, and they play key roles in the post-transcriptional regulation of gene expression. MicroRNAs have recently been found to play a role in linking the innate and adaptive immune systems [40]. It has been shown that microRNAs are regulated by TLR signaling; however, microRNAs can also regulate TLR signaling pathways by targeting TLRs, TLR associated signaling proteins, transcription factors, cytokine mRNAs, and TLR signaling regulators [41]. We will now discuss the microRNAs that are involved in regulating the expression of TLRs, as well as the unconventional roles of microRNAs (Table 3).

### MicroRNAs serve as ligands of TLRs

The conventional role of microRNAs is regulation of gene expression. However, recent studies have suggested that microRNAs act as ligands of TLRs [42, 43]. Since microRNAs are short single-strand RNA molecules, they may bind to the TLRs. MicroRNAs have been suggested to be ligands of TLRs. The activation of nuclear factor κB (NF-κB) by miR-122, miR-15b, miR-21, and miR-155 stimulation can be blocked with TLR1 blocking antibody, while knockdown of TLR1 by shRNA resulted in NK-92 cells losing their capability for miRNA-mediated activation [44].

**Table 1** Toll-like receptors and their ligands

| TLRs | Location | Ligands | Products | Adaptors |
|---|---|---|---|---|
| TLR1 | Plasma membrane | Bacterial triacyl lipopeptides (TLR1/TLR2), miR-122, miR-15b, miR-21, miR-155 | Cytokines | TIR domain-containing adaptor protein (TIRAP), MyD88, Mal (TLR1/TLR2) |
| TLR2 | Plasma membrane | Gram(+) peptidoglycan, lipoteichoic acid, lipoproteins/lipopeptides, glycolipids, mycobacteria lipoarabinomannan<br>Zymosan<br>Pam3CSK4 | Cytokines | TIRAP, MyD88, Mal |
| TLR3 | Endosomes | Virus dsRNA | Cytokines, type 1 IFNs | TRIF |
| TLR4 | Plasma membrane<br>Endosomes | Gram(−)LPS, Taxol, HSP60/70/90<br>Virus glycoproteins<br>Beta-defensin, fibronectin extra domain A, heparan sulfate | Cytokines, type 1 IFNs | TRIF related adaptor molecule (TRAM), TRIF, TIRAP, MyD88, Mal |
| TLR5 | Plasma membrane | Bacteria flagellin | Cytokines | MyD88 |
| TLR6 | Plasma membrane | Diacyl lipopeptide<br>Fungi zymosan (TLR2/TLR6) | Cytokines | TIRAP, MyD88, Mal (TLR2/TLR6) |
| TLR7 | Endosomes | Virus ssRNA, imidazoquinoline, miR-21, Let-7b | Cytokines, type 1 IFNs | MyD88 |
| TLR8 | Endosomes | Virus ssRNA, imidazoquinoline, miR-21, miR-29a | Cytokines, type 1 IFNs | MyD88 |
| TLR9 | Endosomes | Bacteria and mycobacteria CpG DNA<br>CpG oligonucleotides (ODN)<br>HMGB1 | Cytokines, type 1 IFNs | MyD88 |
| TLR10 | Plasma membrane | Lipoteichoic acid | Cytokines | MyD88 |

Another study found that the miR-21 and miR-29a released from cancer cell exosomes can bind to TLR7 in mice and TLR8 in human macrophages [45]. MiR-21 released from tumors can also induce myoblast apoptosis in cancer cachexia via binding to TLR7 and initiating activation of the downstream c-Jun N-terminal kinase pathway [46]. These findings are important for new drug development in cancer treatment. Let-7, a microRNA with a high expression level in microglias and neurons in the central nervous system, was reported to activate TLR7, and the expression level of Let-7b was increased in the cerebrospinal fluid (CSF) of Alzheimer's disease patients [47]. The unconventional role of Let-7b on TLR7 was confirmed by another study [48]. These studies provide evidence that microRNAs can directly bind to TLRs and initiate stimulation of signaling pathways.

### MicroRNAs serve as regulators of TLR mRNA expression

Another direct role of microRNA on TLRs is shown by the fact that the TLR expression level is manipulated by microRNAs. MicroRNAs may control cell differentiation and cell-specific functions through TLRs. A recent study analyzed the expression of TLR2 in rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) following LPS and BLP treatments. Decreased expression of miR-19b was then demonstrated by microRNA microarray, and miR-19a/b was found to directly target TLR2 mRNA and regulate the expression of IL-6 and matrix metalloproteinase 3 release [49]. MiR-105 and miR-143 were also reported to modulate TLR2 protein expression by directly targeting TLR2 mRNA [50, 51]. The relationship between miR-26a and TLR3 was studied in rat macrophages. MiR-26a mimic treatment was shown to inhibit TLR3 expression and improve pristine-induced arthritis in rats [52]. The Let-7 microRNA family was recently reported to regulate TLR4 expression. They showed that overexpression of Let-7e downregulated TLR4 expression, while inhibition of Let-7e could upregulate TLR4 expression [53]. Recently, a group reported that the expression of TLR7 and TLR9 was regulated by miR-126 [54].

The recent discovery of this new role of miRNAs raises interesting questions. For example, why do some miRNAs have dual functions, that is, some miRNAs can silence TLRs through post-transcriptional regulation, while on the other hand, they can serve as ligands and interact directly with TLRs. How is the interaction between the two pathways regulated? What is the role of miRNAs as a TLR ligand in the pathogenesis of disease? Further studies are needed to assess the biological significance of these miRNAs' dual roles and their mechanisms in disease states. Although many questions remain to be solved,

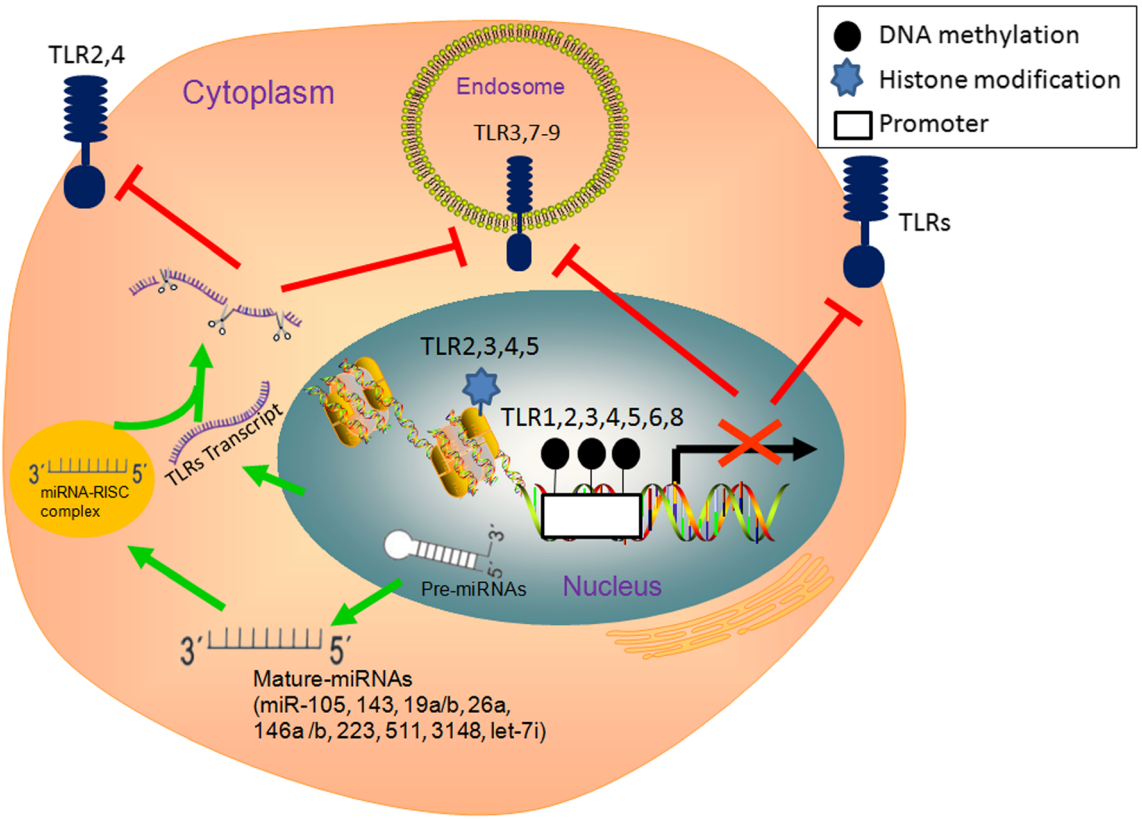


**Fig. 2** Epigenetic regulation of TLRs. TLRs are expressed in both innate and adaptive immune cells. TLRs can be regulated by DNA methylation, histone modifications, and microRNAs, respectively, which will eventually lead to the altered expression of TLRs on the membranes or endosomes. Firstly, DNA methylation that occurs on the promoter region of TLR genes, such as TLR1, 2, 3, 4, 5, 6, and 8, can reduce the expression of TLR on the membranes or endosomes. Secondly, depending on the type of modification, the histone modifications that occurs on the nucleosome near the promoter of TLR genes, such as TLR2, 3, 4, and 5, can also positively or negatively regulate the expression of TLRs on the membranes or endosomes. Finally, TLRs, including TLR2, 3, 4, 7, and 9 can be silenced directly by microRNAs (miRNAs), such as miR-105, 143, 26a, 223, 3148, and let-7i, at the post-transcriptional level. MiRNAs are transcribed from genomic DNA and processed into precursor miRNAs (pre-miRNAs). Pre-miRNAs are then exported to the cytoplasm to generate mature miRNAs. The mature miRNAs are assembled as part of the miRNA-RISC complex, which can silence TLR transcripts through post-transcriptional regulation, and lead to the decreased expression of TLR on the membranes or endosomes

the discovery of this new miRNA function opens up a new and exciting field of miRNA research on TLRs. The unconventional role of microRNA on TLRs is important in that it is a new mechanism of disease pathogenesis as well as a new strategy for disease treatment.

Taken together, epigenetic mechanisms, such as DNA methylation, histone modification, and microRNAs, have provided many new strategies in regulating TLR signaling pathways. There are additional studies that address the epigenetic regulation of Toll-like receptor pathways including TLRs, adaptor proteins, and transcription factors. However, we discussed here the direct regulation of TLRs by these epigenetic mechanisms. DNA methylation, histone modification, and microRNAs may be involved in the pathogenesis of a series of diseases through their interaction with TLRs either directly or indirectly. The study of epigenetic regulation of TLRs may provide new clues to clarify the pathogenesis of autoimmune diseases, including T1D.

## T1D and TLRs

T1D is a T cell-mediated autoimmune disease that is characterized by selective destruction of pancreatic β cells in genetically susceptible individuals [8]. Humoral immunity is involved in the pathogenesis of T1D, and several autoantibodies against β islets have been identified so far. Studies from our group suggest the diagnostic value of autoantibodies in T1D [55–57]. In contrast, cellular immunity has been thought to play a more pivotal role in T1D pathogenesis [58].

Devaraj et al. [59, 60] reported that the surface expression levels of TLR2 and TLR4 were increased in T1D monocytes compared with controls, while the ligands of TLR2 and TLR4, such as endotoxin, Hsp60, and HMGB1, were also increased in T1D patients. These data indicate that the inflammatory response triggered by TLR2 and TLR4 is important in the development of T1D. Our group analyzed the expression of TLR2 and TLR4 in monocytes of T1D patients. However, the

**Table 2** DNA methylation and histone modification of TLRs

| Area of research | Tissue or cell types | Methods of analysis | Main findings | Ref |
|---|---|---|---|---|
| DNA methylation of TLR2 | Healthy human monocytes and macrophages | Bisulfite sequencing | The CpG methylation of the proximal TLR2 promoter is correlated with TLR2 repression | [5] |
| DNA methylation of Tlr6 and Tlr1 | Mouse epiblasts | MeDIP-on-ChiP | During early development, DNA methylation in epiblasts is present in the promoters of the mouse Tlr6 and Tlr1 genes | [30] |
| DNA methylation of TLR5 | Blood and nasal epithelial cells from cystic fibrosis patients | Bisulfite and next-generation sequencing | DNA methylation level was increased at TLR5 promoter | [31] |
| Genome-wide DNA methylation and methylation of TLR8 | Whole blood from post-traumatic stress disorder patients and controls | Bisulfate and HumanMethylation27 BeadChip | An overall increased level of methylation was reported. TLR8 showed increased methylation in patients | [32] |
| Histone modification of TLR2 | Healthy human monocytes | ChIP assays | Histone hyperacetylation and increased promoter access induced TLR2 expression | [33] |
| Histone modification of TLR5 | Human intestinal epithelial cell (IEC) line | ChIP assays | TLR5 expression caused by enhanced histone acetylation at the TLR5 promoter | [34] |
| Histone modification and DNA methylation of TLR4 | Mouse embryonic stem (ES) cells and ES cell-derived differentiated cells | Bisulfate sequencing, methylation sensitive restriction enzymes and ChIP | Analysis of the DNA methylation status of the TLR4 promoter showed increased methylation. ChIP assays showed that histones H3 and H4 are hypoacetylated in ES cells | [35] |
| Histone modification and DNA methylation of TLR4 | Human intestinal epithelia cell(IEC) and monocyte cell line | Bisulfate sequencing and ChIP | Histone deacetylation and DNA methylation in the 5′ region of the TLR4 gene was significantly higher in the LPS-low responder IEC lines than in a monocyte line or an LPS-high responder IEC line | [36] |
| Histone modification and DNA methylation of TLR4 | Human gastric cancer cell lines | Bisulfite and methylation-specific PCR, and ChIP | Recruitment of the MeCP2/HDAC1 repressor complex increased TLR4 expression through DNA methylation and histone modification of the TLR4 promoter | [37] |
| TLR3 expression and histone modification and DNA methylation | Human colon cancer cells (HCT116) | TLR3 ligand Poly I:C, as well as inhibition of HDACs or DNMTs | Poly I:C treatment resulted in a significant increase in TLR3 expression. Inhibition of HDACs or DNMTs prevented the activation of TLR3 signaling pathway | [38] |

**Table 3** Relations between microRNAs and TLRs

| TLRs | microRNAs serve as ligands | microRNAs serve as regulators |
|---|---|---|
| TLR1 | miR-122, miR-15b, miR-21, miR-155 | |
| TLR2 | | miR-19a/b, miR-105, miR-143, miR-146a |
| TLR3 | | miR-26a, miR-223 |
| TLR4 | | Let-7i, miR-223, miR-146a, miR-146b, miR-511 |
| TLR7 | miR-21, Let-7b | miR-3148, miR-126 |
| TLR8 | miR-21, miR-29a | |
| TLR9 | | miR-126 |

results showed that the expression of both TLR2 and TLR4 was not changed in T1D patients compared with controls [61].

Fulminant type 1 diabetes (FT1D) is a subtype of T1D characterized by the abrupt onset of insulin-deficient hyperglycemia [62]. Shibasaki et al. [63] detected the expression of TLR3, TLR7, and TLR9 in the pancreas of FT1D patients by immunohistochemistry and in situ hybridization. Studies from our group found a significant reduction of Foxp3 expression in PBMCs of FT1D patients, which indicated that there may exist a Treg development/function defect. A series of experiments were conducted, and the data suggested that DNA methylation may impair TLR9-induced Foxp3 expression by preventing IRF-7 from binding to the Foxp3 promoter and thus impairing Foxp3 expression [64].

## TLRs induced diabetes in a mouse model

Studies performed on mouse models of type 1 diabetes, especially the non-obese diabetic (NOD) mouse, strongly support the hypothesis that TLRs are involved in the pathogenesis of T1D in NOD mouse.

Vallois et al. [65] found that, compared with the diabetes prone NOD mouse, the expression level of TLR1 in splenocytes and thymocytes from diabetes-resistant Idd6 NOD.C3H-congenic mice was decreased. They suggested that TLR1 pathways are involved in the induction of diabetes in the NOD mouse. Alyanakian et al. [66] found that the oral administration of a bacterial extract (OM-85) can delay the onset of diabetes in NOD mice through TLR2-, TLR4-, and MyD88-dependent signaling pathways. Wen et al. [16] found that upregulation of TLR3 by Poly I:C can lead to diabetes in the B6/RIP-B7.1 mouse, and the underlying mechanism may be related to the upregulated APCs and islet Ag-reactive T cells, which can then destroy islet β cells. In addition, Wen et al. [67] found that MyD88, the key adaptor protein that recognizes microbial stimuli in TLR signaling pathways, was important in the pathogenesis of diabetes because the specific pathogen-free MyD88$^{-/-}$ NOD mice do not develop T1D. Because MyD88 is common to multiple TLRs, further studies were conducted in NOD mice lacking individual TLRs (TLRKO). They found that TLR2, TLR3, and TLR4 were dispensable for the development of T1D when deleted individually. They also found that MyD88 deficiency can change the composition of the gut microbiota [67]. The role of microbiota in the pathogenesis of T1D was also reported to be related to TLRs in several other studies [68, 69].

It has been hypothesized that the TLRs are involved in the balance between CD4$^+$CD25$^+$ T regulatory cells (Tregs) and T effector cells. TLRs may have an effect on autoimmune responses in several ways, such as activation of APCs and T cells [16, 70] and an effect on modulating Tregs [71, 72]. Filippi et al. [73] found that diabetes can be prevented in prediabetic NOD mice after treatment with the TLR2 agonist $Pam_3CSK_4$ (P3C), and they also found an increased number and function of Tregs. An enhanced function of Treg cells through TLR2 was also reported by Karumuthil-Melethil et al. [74]. However, there are several contradictory studies [75, 76]. TLRs are important regulators of Tregs, and it seems that the number and function of Tregs can either be increased or decreased; the concentration of TLR ligands may have a role in influencing the above different outcomes.

## TLRs induced diabetes in a rat model

Bio-breeding diabetes-prone (BBDP) and bio-breeding diabetes-resistant (BBDR) rats are two inbred strains of BB rats, and they are prone to develop diabetes similar to that of human T1D.

Ewel et al. [77] found that a high dose of Poly I:C (a TLR3 agonist) can accelerate the development of diabetes in BBDP rats. However, Sobel et al. [78] found that treatment of BBDP by low doses of Poly I:C (a TLR3 agonist) can prevent diabetes. Sobel et al. [79] also investigated the role of Poly I:C in BBDR rats. They found that both 5 and 10 mg/g body weight can significantly induce the development of diabetes in BBDR rats. It seems that contrary effects of Poly I:C administration are dose-related.

Guberski et al. [80] found that the Kilham rat virus (KRV) can induce diabetes in naive BBDR/Wor rats. However, they did not identify which TLR pathways were involved in the pathogenesis of the disease. Zipris et al. [81, 82] found that the activation of TLRs (TLR2, TLR3, TLR4, TLR7, TLR8, and

TLR9) by Kilham rat virus (KRV) infection can induce T1D in BBDR rats. Recently, Tirabassi et al. [83] used KRV rat cytomegalovirus (RCMV), H-1, vaccinia, Coxsackie B4, and Poly I:C, and they found that KRV and RCMV can induce diabetes in up to 60% of LEW.1WR1 rats, whereas other viruses cannot. Taken together, data obtained from both the mouse and rat models imply that TLRs are involved in the pathogenesis of T1D. Virus infection, TLR ligand activation, and their related pathways may play a pivotal role in mediating TLR-induced diabetes.

### Genetic association of TLRs with T1D

Accumulating genetic association studies also support the hypothesis that TLRs are associated with T1D in humans. Considering the role of TLRs in mediating the link between the environment factors and the adaptive immune system, Pirie et al. [84] assessed the role of TLR3 in South African T1D patients. They found a significant association between TLR3 and T1D. However, the results were no longer significant after correction of the *P* value [84]. Assmann et al. [85] found that rs3775291 and rs13126816 polymorphisms in TLR3 were associated with risk for T1D, while rs5743313 and rs11721827 polymorphisms were associated with age at T1D diagnosis and poor glycemic control. They also found that the number of risk alleles seemed to influence the risk for T1D, which suggests that these polymorphisms might interact in the susceptibility to T1D [85]. Park et al. [86] investigated the association of TLR2 in Korean T1D patients, and they found that TLR2 polymorphisms were associated with T1D and the differences were not influenced by HLA genes. The authors suggested a close relationship between innate and adaptive immunity. The results were confirmed by Bjørnvold et al. [87] in a Norwegian population. However, there are also studies that have demonstrated no association of TLR2 and TLR4 polymorphisms with T1D [88, 89]. Sun et al. [90] genotyped 28 SNPs in TLR1–6 and TLR8–9 in 429 T1D patients and 300 controls, and they found that SNPs and haplotypes in TLR1 and TLR6 were associated with T1D in a Chinese population. However, it should be noted that the sample size in some of the studies was rather small.

Given that the SNPs in TLRs may change the expression, cell surface trafficking, and functional responses level of TLRs [91, 92], these genetic association studies are important in support of the view that some TLRs may be involved in the pathogenesis of T1D. However, all of these association studies failed to genotype all SNPs in the reported TLR genes. Thus, the TLR allele or the haplotype may not have been accurately reconstructed. Further studies performed in different populations are needed to explore the possible association between TLRs and T1D.

## Conclusions/summary

T1D is an autoimmune disease [93]. As a component of innate immunity, TLRs are believed to be involved in the pathogenesis of autoimmune diseases including T1D. Indeed, results from NOD mouse model and rat model studies, genetic association studies, and TLR expression studies in T1D patients all support this view. Epigenetics has emerged as a new mechanism in regulating TLR expression. DNA methylation, histone modification, and microRNAs can directly regulate the expression of TLRs and thus influence TLR signaling pathways. The reversible nature of epigenetic alterations allows epigenetic regulators of TLRs as potential options for T1D therapy. Some therapeutic strategies have been proposed [94–97]. However, the conflicting findings in animal models suggest that TLRs may act as a double-edged sword. Their safety and efficacy should be well considered before applying them to the treatment of T1D. Many more studies of TLRs should be performed to clarify the underlying epigenetic mechanisms of TLR regulation and the role of TLRs in the pathogenesis, prevention, and treatment of T1D. Further studies are still needed to demonstrate the role of epigenetic regulation of TLRs in the pathogenesis of T1D.

**Funding information** This work was supported by grants from the National Key Research and Development Program of China (2016YFC1305000), the National Natural Science Foundation of China (No. 81400783), the National Key Technology R&D program (2015BAI12B13), and the strategic forerunner project of Central South University (ZLXD2016003).

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Medzhitov R (2007) Recognition of microorganisms and activation of the immune response. Nature 449:819–826
2. Barton GM, Medzhitov R (2003) Toll-like receptor signaling pathways. Science 300:1524–1525
3. Cook DN, Pisetsky DS, Schwartz DA (2004) Toll-like receptors in the pathogenesis of human disease. Nat Immunol 5:975–979
4. Qian C, Cao X (2013) Regulation of Toll-like receptor signaling pathways in innate immune responses. Ann N Y Acad Sci 1283:67–74
5. Haehnel V, Schwarzfischer L, Fenton MJ, Rehli M (2002) Transcriptional regulation of the human toll-like receptor 2 gene in monocytes and macrophages. J Immunol 168:5629–5637
6. Morse ZJ, Horwitz MS (2017) Innate viral receptor signaling determines type 1 diabetes onset. Front Endocrinol (Lausanne) 8:249. https://doi.org/10.3389/fendo.2017.00249
7. Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. Lancet 383:69–82

8. Xie Z, Chang C, Zhou Z (2014) Molecular mechanisms in autoimmune type 1 diabetes: a critical review. Clin Rev Allergy Immunol 47:174–192
9. Liu Y, Yin H, Zhao M, Lu Q (2014) TLR2 and TLR4 in autoimmune diseases: a comprehensive review. Clin Rev Allergy Immunol 47:136–147
10. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86:973–983
11. Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A (2005) The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci U S A 102:9577–9582
12. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650
13. Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G (2002) Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168:4531–4537
14. Muzio M, Bosisio D, Polentarutti N, D'Amico G, Stoppacciaro A, Mancinelli R, van't Veer C, Penton-Rol G, Ruco LP, Allavena P, Mantovani A (2000) Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol 164:5998–6004
15. Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J (2003) Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. J Exp Med 197:403–411
16. Wen L, Peng J, Li Z, Wong FS (2004) The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets. J Immunol 172:3173–3180
17. Zarember KA, Godowski PJ (2002) Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol 168:554–561
18. Meylan E, Tschopp J, Karin M (2006) Intracellular pattern recognition receptors in the host response. Nature 442:39–44
19. Alisi A, Carsetti R, Nobili V (2011) Pathogen- or damage-associated molecular patterns during nonalcoholic fatty liver disease development. Hepatology 54:1500–1502
20. Carty M, Goodbody R, Schroder M, Stack J, Moynagh PN, Bowie AG (2006) The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. Nat Immunol 7:1074–1081
21. O'Neill LA, Bowie AG (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 7:353–364
22. Brodsky I, Medzhitov R (2007) Two modes of ligand recognition by TLRs. Cell 130:979–981
23. Kawai T, Akira S (2007) Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med 13:460–469
24. Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499–511
25. Zipris D (2008) Innate immunity and its role in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes 15:326–331
26. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S (2003) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301:640–643
27. Montero Vega MT, de Andres Martin A (2009) The significance of toll-like receptors in human diseases. Allergol Immunopathol (Madr) 37:252–263
28. Liew FY, Xu D, Brint EK, O'Neill LA (2005) Negative regulation of toll-like receptor-mediated immune responses. Nat Rev Immunol 5:446–458
29. Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45
30. Borgel J, Guibert S, Li Y, Chiba H, Schubeler D, Sasaki H, Forne T, Weber M (2010) Targets and dynamics of promoter DNA methylation during early mouse development. Nat Genet 42:1093–1100
31. Magalhaes M, Rivals I, Claustres M, Varilh J, Thomasset M, Bergougnoux A, Mely L, Leroy S, Corvol H, Guillot L, Murris M, Beyne E, Caimmi D, Vachier I, Chiron R, De Sario A (2017) DNA methylation at modifier genes of lung disease severity is altered in cystic fibrosis. Clin Epigenetics 9:19. https://doi.org/10.1186/s13148-016-0300-8
32. Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley B, Tang Y, Gillespie CF, Cubells JF, Ressler KJ (2011) Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. Am J Med Genet B Neuropsychiatr Genet 156B:700–708
33. Johnson CM, Tapping RI (2007) Microbial products stimulate human Toll-like receptor 2 expression through histone modification surrounding a proximal NF-kappaB-binding site. J Biol Chem 282:31197–31205
34. Thakur BK, Dasgupta N, Ta A, Das S (2016) Physiological TLR5 expression in the intestine is regulated by differential DNA binding of Sp1/Sp3 through simultaneous Sp1 dephosphorylation and Sp3 phosphorylation by two different PKC isoforms. Nucleic Acids Res 44:5658–5672
35. Zampetaki A, Xiao Q, Zeng L, Hu Y, Xu Q (2006) TLR4 expression in mouse embryonic stem cells and in stem cell-derived vascular cells is regulated by epigenetic modifications. Biochem Biophys Res Commun 347:89–99
36. Takahashi K, Sugi Y, Hosono A, Kaminogawa S (2009) Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis. J Immunol 183:6522–6529
37. Kim TW, Lee SJ, Oh BM, Lee H, Uhm TG, Min JK, Park YJ, Yoon SR, Kim BY, Kim JW, Choe YK, Lee HG (2016) Epigenetic modification of TLR4 promotes activation of NF-kappaB by regulating methyl-CpG-binding domain protein 2 and Sp1 in gastric cancer. Oncotarget 7:4195–4209
38. McKernan DP, Hennessy C (2017) Epigenetic modifications influence TLR3 expression and activity. FASEB J 31:1060.1065–1060.1065
39. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Roder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Derrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foissac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Robyr D, Sammeth M, Schaffer L, See LH, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigo R, Gingeras TR (2012) Landscape of transcription in human cells. Nature 489:101–108
40. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D (2010) Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol 10:111–122
41. O'Neill LA, Sheedy FJ, McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol 11:163–175

42. Fabbri M, Paone A, Calore F, Galli R, Croce CM (2013) A new role for microRNAs, as ligands of Toll-like receptors. RNA Biol 10: 169–174
43. Olivieri F, Rippo MR, Prattichizzo F, Babini L, Graciotti L, Recchioni R, Procopio AD (2013) Toll like receptor signaling in "inflammaging": microRNA as new players. Immun Ageing 10:11
44. He S, Chu J, Wu LC, Mao H, Peng Y, Alvarez-Breckenridge CA, Hughes T, Wei M, Zhang J, Yuan S, Sandhu S, Vasu S, Benson DM Jr, Hofmeister CC, He X, Ghoshal K, Devine SM, Caligiuri MA, Yu J (2013) MicroRNAs activate natural killer cells through Toll-like receptor signaling. Blood 121:4663–4671
45. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D, Croce CM (2012) MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci U S A 109:E2110–E2116
46. He WA, Calore F, Londhe P, Canella A, Guttridge DC, Croce CM (2014) Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7. Proc Natl Acad Sci U S A 111:4525–4529
47. Lehmann SM, Kruger C, Park B, Derkow K, Rosenberger K, Baumgart J, Trimbuch T, Eom G, Hinz M, Kaul D, Habbel P, Kalin R, Franzoni E, Rybak A, Nguyen D, Veh R, Ninnemann O, Peters O, Nitsch R, Heppner FL, Golenbock D, Schott E, Ploegh HL, Wulczyn FG, Lehnardt S (2012) An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. Nat Neurosci 15:827–835
48. Park CK, Xu ZZ, Berta T, Han Q, Chen G, Liu XJ, Ji RR (2014) Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron 82:47–54
49. Philippe L, Alsaleh G, Suffert G, Meyer A, Georgel P, Sibilia J, Wachsmann D, Pfeffer S (2012) TLR2 expression is regulated by microRNA miR-19 in rheumatoid fibroblast-like synoviocytes. J Immunol 188:454–461
50. Benakanakere MR, Li Q, Eskan MA, Singh AV, Zhao J, Galicia JC, Stathopoulou P, Knudsen TB, Kinane DF (2009) Modulation of TLR2 protein expression by miR-105 in human oral keratinocytes. J Biol Chem 284:23107–23115
51. Guo H, Chen Y, Hu X, Qian G, Ge S, Zhang J (2013) The regulation of Toll-like receptor 2 by miR-143 suppresses the invasion and migration of a subset of human colorectal carcinoma cells. Mol Cancer 12:77
52. Jiang C, Zhu W, Xu J, Wang B, Hou W, Zhang R, Zhong N, Ning Q, Han Y, Yu H, Sun J, Meng L, Lu S (2014) MicroRNA-26a negatively regulates toll-like receptor 3 expression of rat macrophages and ameliorates pristane induced arthritis in rats. Arthritis Res Ther 16:R9
53. Androulidaki A, Iliopoulos D, Arranz A, Doxaki C, Schworer S, Zacharioudaki V, Margioris AN, Tsichlis PN, Tsatsanis C (2009) The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. Immunity 31:220–231
54. Agudo J, Ruzo A, Tung N, Salmon H, Leboeuf M, Hashimoto D, Becker C, Garrett-Sinha LA, Baccarini A, Merad M, Brown BD (2014) The miR-126-VEGFR2 axis controls the innate response to pathogen-associated nucleic acids. Nat Immunol 15:54–62
55. Huang G, Xiang Y, Pan L, Li X, Luo S, Zhou Z (2013) Zinc transporter 8 autoantibody (ZnT8A) could help differentiate latent autoimmune diabetes in adults (LADA) from phenotypic type 2 diabetes mellitus. Diabetes Metab Res Rev 29:363–368
56. Zhou Z, Xiang Y, Ji L, Jia W, Ning G, Huang G, Yang L, Lin J, Liu Z, Hagopian WA, Leslie RD, Group LCS (2013) Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. Diabetes 62:543–550
57. Liu L, Li X, Xiang Y, Huang G, Lin J, Yang L, Zhao Y, Yang Z, Hou C, Li Y, Liu J, Zhu D, Leslie RD, Wang X, Zhou Z, Group LCS (2015) Latent autoimmune diabetes in adults with low-titer GAD antibodies: similar disease progression with type 2 diabetes: a nationwide, multicenter prospective study (LADA China study 3). Diabetes Care 38:16–21
58. Lehuen A, Diana J, Zaccone P, Cooke A (2010) Immune cell crosstalk in type 1 diabetes. Nat Rev Immunol 10:501–513
59. Devaraj S, Dasu MR, Park SH, Jialal I (2009) Increased levels of ligands of Toll-like receptors 2 and 4 in type 1 diabetes. Diabetologia 52:1665–1668
60. Devaraj S, Dasu MR, Rockwood J, Winter W, Griffen SC, Jialal I (2008) Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. J Clin Endocrinol Metab 93:578–583
61. Du T, Zhou ZG, You S, Lin J, Yang L, Zhou WD, Huang G, Chao C (2009) Regulation by 1, 25-dihydroxy-vitamin D3 on altered TLRs expression and response to ligands of monocyte from autoimmune diabetes. Clin Chim Acta 402:133–138
62. Zheng C, Zhou Z, Yang L, Lin J, Huang G, Li X, Zhou W, Wang X, Liu Z (2011) Fulminant type 1 diabetes mellitus exhibits distinct clinical and autoimmunity features from classical type 1 diabetes mellitus in Chinese. Diabetes Metab Res Rev 27:70–78
63. Shibasaki S, Imagawa A, Tauriainen S, Iino M, Oikarinen M, Abiru H, Tamaki K, Seino H, Nishi K, Takase I, Okada Y, Uno S, Murase-Mishiba Y, Terasaki J, Makino H, Shimomura I, Hyoty H, Hanafusa T (2010) Expression of toll-like receptors in the pancreas of recent-onset fulminant type 1 diabetes. Endocr J 57:211–219
64. Wang Z, Zheng Y, Hou C, Yang L, Li X, Lin J, Huang G, Lu Q, Wang CY, Zhou Z (2013) DNA methylation impairs TLR9 induced Foxp3 expression by attenuating IRF-7 binding activity in fulminant type 1 diabetes. J Autoimmun 41:50–59
65. Vallois D, Grimm CH, Avner P, Boitard C, Rogner UC (2007) The type 1 diabetes locus Idd6 controls TLR1 expression. J Immunol 179:3896–3903
66. Alyanakian MA, Grela F, Aumeunier A, Chiavaroli C, Gouarin C, Bardel E, Normier G, Chatenoud L, Thieblemont N, Bach JF (2006) Transforming growth factor-beta and natural killer T-cells are involved in the protective effect of a bacterial extract on type 1 diabetes. Diabetes 55:179–185
67. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV (2008) Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature 455: 1109–1113
68. Paun A, Yau C, Danska JS (2017) The influence of the microbiome on type 1 diabetes. J Immunol 198:590–595
69. Yiu JH, Dorweiler B, Woo CW (2017) Interaction between gut microbiota and toll-like receptor: from immunity to metabolism. J Mol Med (Berl) 95:13–20
70. Amiset L, Fend L, Gatard-Scheikl T, Rittner K, Duong V, Rooke R, Muller S, Bonnefoy JY, Preville X, Haegel H (2012) TLR2 ligation protects effector T cells from regulatory T-cell mediated suppression and repolarizes T helper responses following MVA-based cancer immunotherapy. Oncoimmunology 1:1271–1280
71. Wong FS, Wen L (2008) Toll-like receptors and diabetes. Ann N Y Acad Sci 1150:123–132
72. Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK (2005) Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+CD25+ T regulatory cells. J Immunol 175:8051–8059
73. Filippi CM, Ehrhardt K, Estes EA, Larsson P, Oldham JE, von Herrath MG (2011) TLR2 signaling improves immunoregulation to prevent type 1 diabetes. Eur J Immunol 41:1399–1409

74. Karumuthil-Melethil S, Perez N, Li R, Vasu C (2008) Induction of innate immune response through TLR2 and dectin 1 prevents type 1 diabetes. J Immunol 181:8323–8334
75. Chen Q, Davidson TS, Huter EN, Shevach EM (2009) Engagement of TLR2 does not reverse the suppressor function of mouse regulatory T cells, but promotes their survival. J Immunol 183:4458–4466
76. Al Shamsi M, Shahin A, Iwakura Y, Lukic ML, Mensah-Brown EP (2013) Pam3CSK(4) enhanced beta cell loss and diabetogenesis: the roles of IFN-gamma and IL-17. Clin Immunol 149:86–96
77. Ewel CH, Sobel DO, Zeligs BJ, Bellanti JA (1992) Poly I:C accelerates development of diabetes mellitus in diabetes-prone BB rat. Diabetes 41:1016–1021
78. Sobel DO, Goyal D, Ahvazi B, Yoon JW, Chung YH, Bagg A, Harlan DM (1998) Low dose poly I:C prevents diabetes in the diabetes prone BB rat. J Autoimmun 11:343–352
79. Sobel DO, Newsome J, Ewel CH, Bellanti JA, Abbassi V, Creswell K, Blair O (1992) Poly I:C induces development of diabetes mellitus in BB rat. Diabetes 41:515–520
80. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM (1991) Induction of type I diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. Science 254:1010–1013
81. Zipris D, Lien E, Nair A, Xie JX, Greiner DL, Mordes JP, Rossini AA (2007) TLR9-signaling pathways are involved in Kilham rat virus-induced autoimmune diabetes in the biobreeding diabetes-resistant rat. J Immunol 178:693–701
82. Zipris D, Lien E, Xie JX, Greiner DL, Mordes JP, Rossini AA (2005) TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. J Immunol 174:131–142
83. Tirabassi RS, Guberski DL, Blankenhorn EP, Leif JH, Woda BA, Liu Z, Winans D, Greiner DL, Mordes JP (2010) Infection with viruses from several families triggers autoimmune diabetes in LEW*1WR1 rats: prevention of diabetes by maternal immunization. Diabetes 59:110–118
84. Pirie FJ, Pegoraro R, Motala AA, Rauff S, Rom L, Govender T, Esterhuizen TM (2005) Toll-like receptor 3 gene polymorphisms in South African Blacks with type 1 diabetes. Tissue Antigens 66: 125–130
85. Assmann TS, Brondani Lde A, Bauer AC, Canani LH, Crispim D (2014) Polymorphisms in the TLR3 gene are associated with risk for type 1 diabetes mellitus. Eur J Endocrinol 170:519–527
86. Park Y, Park S, Yoo E, Kim D, Shin H (2004) Association of the polymorphism for Toll-like receptor 2 with type 1 diabetes susceptibility. Ann N Y Acad Sci 1037:170–174
87. Bjørnvold M, Munthe-Kaas MC, Egeland T, Joner G, Dahl-Jorgensen K, Njolstad PR, Akselsen HE, Gervin K, Carlsen KC, Carlsen KH, Undlien DE (2009) A TLR2 polymorphism is associated with type 1 diabetes and allergic asthma. Genes Immun 10: 181–187
88. Santin I, Bilbao JR, de Nanclares GP, Calvo B, Castano L (2006) No association of TLR2 and TLR4 polymorphisms with type I diabetes mellitus in the Basque population. Ann N Y Acad Sci 1079:268–272
89. Dezsofi A, Szebeni B, Hermann CS, Kapitany A, Veres G, Sipka S, Korner A, Madacsy L, Korponay-Szabo I, Rajczy K, Arato A (2008) Frequencies of genetic polymorphisms of TLR4 and CD14 and of HLA-DQ genotypes in children with celiac disease, type 1 diabetes mellitus, or both. J Pediatr Gastroenterol Nutr 47:283–287
90. Sun C, Zhi D, Shen S, Luo F, Sanjeevi CB (2014) SNPs in the exons of Toll-like receptors are associated with susceptibility to type 1 diabetes in Chinese population. Hum Immunol 75:1084–1088
91. Uciechowski P, Imhoff H, Lange C, Meyer CG, Browne EN, Kirsten DK, Schroder AK, Schaaf B, Al-Lahham A, Reinert RR, Reiling N, Haase H, Hatzmann A, Fleischer D, Heussen N, Kleines M, Rink L (2011) Susceptibility to tuberculosis is associated with TLR1 polymorphisms resulting in a lack of TLR1 cell surface expression. J Leukoc Biol 90:377–388
92. Johnson CM, Lyle EA, Omueti KO, Stepensky VA, Yegin O, Alpsoy E, Hamann L, Schumann RR, Tapping RI (2007) Cutting edge: a common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. J Immunol 178:7520–7524
93. Eisenbarth GS (1986) Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314:1360–1368
94. Bednar KJ, Ridgway WM (2014) Targeting innate immunity for treatment of type 1 diabetes. Immunotherapy 6:1239–1242
95. Needell JC, Zipris D (2017) Targeting innate immunity for type 1 diabetes prevention. Curr Diab Rep 17:113. https://doi.org/10.1007/s11892-017-0930-z
96. Bednar KJ, Tsukamoto H, Kachapati K, Ohta S, Wu Y, Katz JD, Ascherman DP, Ridgway WM (2015) Reversal of new-onset type 1 diabetes with an agonistic TLR4/MD-2 monoclonal antibody. Diabetes 64:3614–3626
97. Hara N, Alkanani AK, Dinarello CA, Zipris D (2014) Histone deacetylase inhibitor suppresses virus-induced proinflammatory responses and type 1 diabetes. J Mol Med (Berl) 92:93–102