Chapter 1

The Innate Immune System via Toll-Like Receptors (TLRs) in Type 1 Diabetes - Mechanistic Insights

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Additional information is available at the end of the chapter

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Abstract

Type 1 diabetes (T1D) is a form of diabetes mellitus resulting from the lack of insulin secretion by the pancreatic beta cells and which accounts for approximately 5% of the total number of patients with diabetes worldwide. T1D is one of the most common endocrine disorders of children, and its incidence is steadily increasing. T1D is largely considered an autoimmune disorder resulting from the specific destruction of the pancreatic beta-cells that produce insulin. However, T1D pathophysiology is still not completely understood, and although insulin and other therapies ameliorate the manifestations of the disease, no cure is currently available. Traditionally, T1D has been thought of as a condition of cellular adaptive immunity, but evidence exists that components of the innate immune system, such as Toll-like receptors (TLRs), play a critical role in T1D development. TLRs have a central role in sensing microbial infections as well as endogenous alarm signals and trigger the release of inflammatory cytokines. The involvement of these receptors in the pathophysiology of several chronic diseases has become a major research interest, and in the last two decades, many studies have suggested the involvement of the innate immune system in the mechanism triggering T1D. Furthermore, microvascular complications in diabetic patients result in considerable morbidity, particularly diabetic nephropathy, retinopathy, and atherosclerosis. A hallmark of diabetic vascular pathology is inflammation and endothelial dysfunction. Recent literature suggests that TLR signaling is involved in vascular inflammation and endothelial dysfunction and that TLR activation may play a crucial role in diabetic microangiopathy. However, the mechanisms by which TLRs and their ligands contribute to T1D are not yet clear, and further investigation is needed. The goal of the present chapter is to address the contribution of TLRs to the mechanisms leading to the development and progression of T1D and to review current possibilities of targeting TLRs to forestall diabetic complications.

Keywords: Toll-like receptors, type 1 diabetes, DAMPS, innate immune system, microangiopathy

1. Introduction

The innate immune system is the first line of defense against invading organisms and other dangerous events in our body. Unlike the acquired immune system, innate immunity identifies the presence of harm via pattern recognition receptors (PRRs). Toll-like receptors (TLRs) are one of the most important classes of PRRs for sensing harmful signals. TLRs can recognize two types of molecules: (1) conserved pathogen molecules such as lipopolysaccharide (LPS), proteins, and nucleic acids expressed by microbes, viruses, bacteria, and fungi, which are known as pathogen-associated molecular patterns or PAMPS [1-2] and (2) endogenous molecules released from damaged cells or tissues such as HMGB-1, HSP60, and C-reactive protein called damage-associated patterns or DAMPS [3]. To date, ten TLRs have been identified in humans (TLR1–TLR10) and twelve in mice (TLR1–TLR9 and TLR11–TLR13). Most TLRs are located on the cell surface, except for TLR3, TLR7, TLR8, and TLR9, which are expressed in the intracellular compartment, the endosome [4].

All TLRs share their intracellular domain with the interleukin-1-receptor (IL-1R) family. Two major intracellular signaling pathways are triggered by TLRs, one that is canonical and dependent on myeloid differentiation primary response protein 88 (MyD88) and another that is noncanonical and MyD88-independent (Figure 1) pathway. MyD88 binds to TLRs upon activation and is essential for the induction of inflammatory cytokines via TLRs. All TLRs, except TLR3, can activate a MyD88-dependent pathway, which involves mitogen-activated kinases and leads to the transcription of pro-inflammatory genes through the activation of nuclear factor κB (NF-κB). TLR3 activates the TRIF-mediated pathway, a MyD88-independent pathway that in turn activates interferon regulatory factor 3 (IRF-3), inducing the expression of interferons (IFNs) [5]. TLR pathway activation results in the activation of the inhibitor of NF-κB kinase (IKK) complex and the transcription factor nuclear factor κB (NF-κB). NF-κB has been extensively studied as a regulator of inflammatory mediators, including tumor necrosis factor alpha (TNF-α). Increased levels of interleukin 1 beta (IL-1β) and TNF-α have been correlated with an expression of TLR2 and TLR4 on monocytes from T1D patients [6] (Figure 1).

Type 1 diabetes (T1D) is a disease in which the pancreatic insulin-producing beta-cells are lost or destroyed, usually via autoimmune mechanisms. Consequently, slow and progressive islet beta-cell impairment and total loss of insulin secretion are observed [7-8]. How the disease is triggered is unknown; however, research in animal models of T1D supports the hypothesis that microbial infection and/or innate immune system activation play an important role in disease mechanisms [9]. Several lines of evidence suggest that T1D progression is strongly heritable [10-11]. However, in addition to genetic factors, environmental factors such as chemicals, infections, and components of early childhood diet might contribute to T1D onset [12]. The development of T1D can be classified into two stages. In the first stage, called insulitis, Langerhans islets in the pancreas are progressively infiltrated by cells of the immune system, especially T cells and macrophages (Figure 2). In the second stage, most beta-cells are destroyed by the infiltrating immune cells. Apoptosis of pancreatic beta-cells is the last step in the initial pathogenesis of T1D [13]. Regardless of the progress toward the last step of T1D development,

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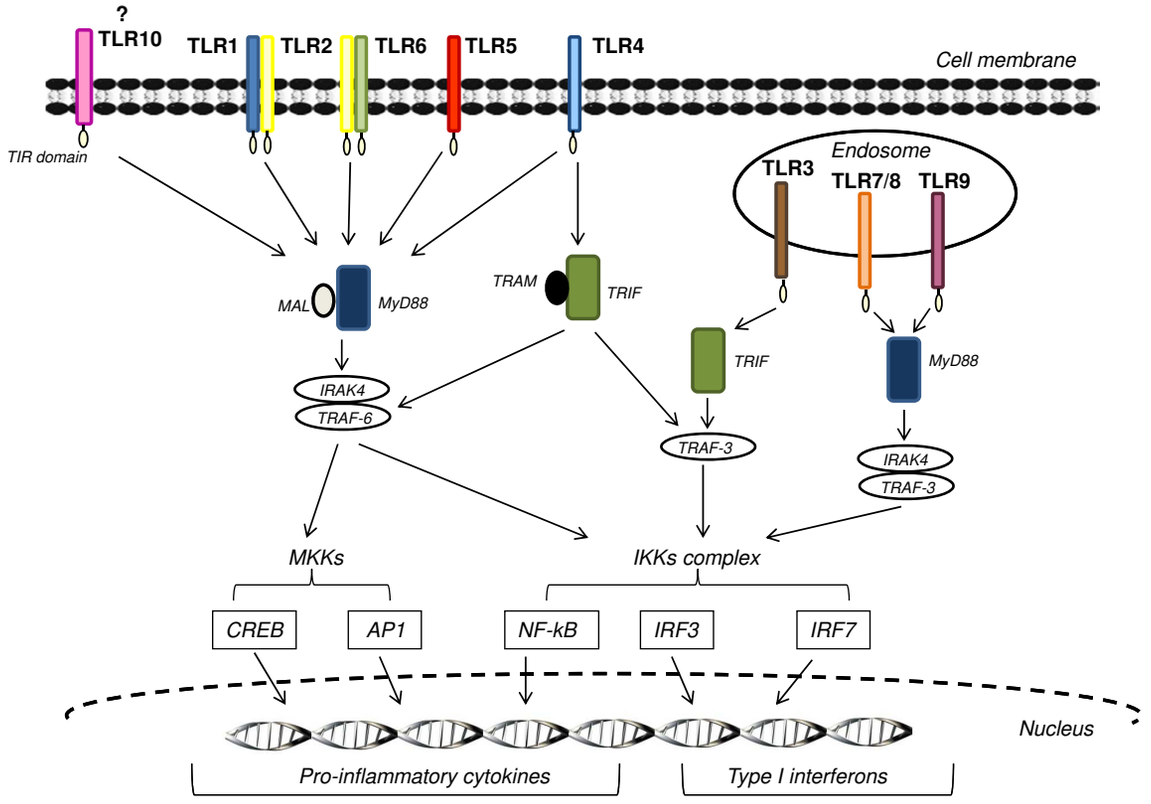

Figure 1. Ten human TLRs and their pathways. Signaling pathways activated by TLRs might be MyD88 dependent or independent. MyD88 is an adaptor molecule, which recruits IRAK and induces phosphorylation. IRAK associates with TRAF6 or TRAF3, leading to the activation of IKK complex or MKKs and resulting in the activation of NF-κB and other important transcription factors (CREB and AP1). The activation of a MyD88-dependent pathway leads to induction of inflammatory cytokines or type I interferons (IFNs). All TLRs, except TLR3, can activate MyD88. The MyD88-independent pathway is called TRIF-mediated pathway (TIR-domain-containing adaptor inducing an NF-κB). TLR4 can also utilize the TRIF-related adaptor molecule (TRAM) for the activation of NF-κB. In order to switch signaling from MyD88 to TRIF, TLR4 moves from plasma membrane to the endosomes. Little is known about TLR10 and its ligands, but this receptor may heterodimerize with TLR1 and TLR2. The activation of TLRs especially TLR2 and TLR4 pathways leads to complications that are associated with the pathogenesis of diabetes. CRBE: cyclic AMP-responsive element binding-protein; AP1: activator protein 1.

the initial step, triggering anti-islet autoimmunity, is still unclear and this is one of the most relevant issues in the field of autoimmune diseases.

Generally, TLR-expressing innate immune cells trigger the initial actions against dangerous signals, which later lead to the activation of T and B cells of the adaptive immune system. Although the primary function of TLRs is linked to the innate immunity, there are no reasons why TLRs may not have a direct function on adaptive immunity, and it has been recently demonstrated that TLRs are also expressed not only in cells from the innate immune but also in T and B cells [14-16]. Continuous release of DAMPs from damaged cells and tissues may maintain the activation of the innate immune system in diseases with long-term low-level inflammation. Therefore, the participation of innate immunity via TLRs not only in acute but also in chronic disease has been recently speculated in the literature. The expression of TLRs in T or B cells has been suggested to provide a cell intrinsic mechanism for innate signals

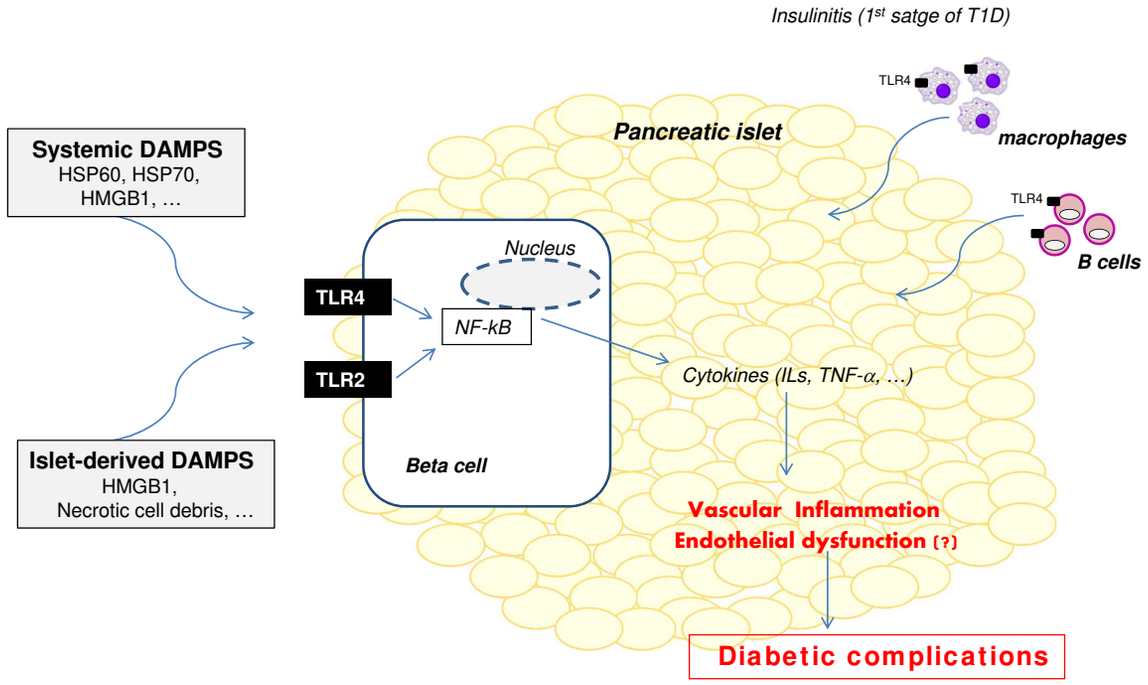

Figure 2. Involvement of TLR2 and TLR4 in the development of T1D. TLR2 and TLR4 on islet beta-cells senses expression changes in DAMPS such as HMGB1 and contribute to the initiation of T1D. Activation of TLR2 and TLR4 leads to NF-κB activation and pro-inflammatory cytokines production, which play a part in T1D inflammatory process and possibly endothelial dysfunction resulting in diabetic vascular complications. In addition, in T1D, the pancreas is progressively infiltrated by cells of the immune system such as macrophages and B-cells which express TLR2 and TLR4.

regulating adaptive immune responses [3, 16], which suggest the TLR-mediated activation of innate immunity may be controlling chronic disorders. However, the exact role of the innate immunity and TLRs in chronic diseases such as T1D is still under discussion.

TLRs play a major role in the development of several pancreatic diseases [17]. In the last decade, the involvement of the innate immune system in diabetes development and complications has been highlighted and investigated by many authors [18-19]. The idea that the initial event in the pathogenesis of autoimmune T1D comprises sensing of molecular patterns from apoptotic beta cells by TLRs has been suggested in recent papers [8, 20]. In T1D, necrotic beta-cells might stimulate dendritic cells (DCs), which are essential in defending against microbial infections and are involved in initiating and regulating immune responses linked to inflammation [21]. In addition, during the development of T1D, multiple interactions occur between DCs, macrophages, natural killer cells (NKs), and lymphocytes. Ultimately, the activation of these cells leads to induction of inflammatory genes [22]. A proinflammatory state is characteristic of T1D and is manifested by elevated circulating and cellular biomarkers such as augmented plasma levels of C-reactive proteins (CRP), cytokines (IL-1B, TNF, and IL-6), soluble cell protein adhesion, chemokines, etc. These increases are further accentuated in T1D patients with vascular complications [23-24].

Of the various TLRs, TLR2 and TLR4 have an important role in inflammation associated with diabetes. In addition, T1D harbors a considerably elevated risk for progressive atherosclerotic

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events and TLRs may be involved, but the mechanistic basis for this phenomenon is not completely clear. Likewise, TLRs are involved in the pathogenesis of diabetic microvascular alterations [25]. However, the TLR activation in this diabetic condition and its association with vascular or endothelial dysfunction has not been well characterized. Experimental studies have shown that TLR2 and TLR4 could be important participants in the progression of atherosclerosis in diabetes [6, 26-27]. On the other hand, TLR3, TLR7, and TLR9 seem to be involved in the initiation of TD1 [5, 28].

In this chapter, based on the recent advances in understanding the role of the innate immune system in chronic disease, we focus on the contribution of TLRs to the mechanisms that trigger T1D onset and the development of its complications. This information might provide new insights into possibilities for therapeutic intervention by targeting and modulating the immune system to abrogate or prevent T1D.

2. Contribution of TLRs in the pathogenesis of T1D

Recently, it has become evident that the dysregulation of the innate immune system can precipitate autoimmune diseases, including T1D. Given its essential role in orchestrating innate immune responses, the TLRs may be expected to play a significant role in the T1D development, progression, and its complications. The connections among inflammation, hyperglycemia, and diabetes have clear implications for the immune system. In addition, TLRs activate two types of downstream signaling pathways that lead to the activation of NF-κB with concomitant increase in inflammatory cytokine secretion (Figure 1). Both pathways contribute significantly to the pathophysiology of inflammation in endothelial dysfunction and are relevant to diabetic microangiopathy. Therefore, the key point regarding the involvement of TLRs in T1D and its complications seems to be the inflammatory process.

2.1. TLR2 and TLR4

The activation of the innate immune system via TLRs, in particular, TLR2 and TLR4, seems to play an important role in the development of T1D. Many authors proposed the sensing of DAMPs released from damaged pancreatic β-cells by TLR2 to be first event in the development of T1D [29-30]. The Increased expression of TLR2 and TLR4 in monocytes was described in patients with T1D compared to healthy patients [6]. In addition, the expression of TLR2 is augmented in T1D in both rat and human kidneys and has been associated with vascular complications [31]. Furthermore, T1D patients with microvascular complications showed increases in TLR2 and TLR4 activity in monocytes compared with matched controls [25]. The higher expression of TLR2 and TLR4 is associated with poor glycemic control, while the knockdown of both TLR2 and TLR4 resulted in a 76% decrease in a high glucose-induced NF-κB response, suggesting an additive effect [32-33]. Also, it has been demonstrated that deletion of TLR2 in mice significantly abrogates the proinflammatory state of T1D for up to 14 weeks in mice and improves the wound healing process, supporting a role for TLR2 in promoting inflammation in diabetes [30, 34].

There are ample data supporting an important role for inflammation associated with atherosclerosis in T1D and TLRs may be mediating this process. A recent study demonstrated that TLR2 and TLR4 mediate inflammatory pathways in endothelial cells exposed to high glucose [35], although the precise mechanism by which glucose fluctuations mediate inflammation in endothelial dysfunction is unknown. In apolipoprotein $E^{-/-}$ mice, the deficiency of IP-10 (interferon-gamma-inducible-protein 10) or its receptor (CXCR3) reduces vascular lesion formation. Also, elevated serum IP-10 levels have been shown in diabetes as well as increased monocytic IP-10 in T1D patients, but it is unclear if the patients had complications [36]. TLR4 agonists such as LPS have been shown to induce IP-10 production, and it has been demonstrated that down-regulation of TLR2 and TLR4 abrogates high glucose-induced IP-10 release via NF-κB inhibition [32].

Although many studies have highlighted the involvement of TLRs in the pathogenesis of T1D, TLRs might also have a beneficial role against T1D. Since the cause of T1D and the mechanisms involving this condition are not completely elucidated, the contradiction about the role of the TLRs in T1D could be dependent on the disease stage or how the disease was triggered. T cells, especially CD4 and CD25 T cells, play an important role in the prevention of autoimmunity. These cells not only express different TLRs, including TLR2, but are also functionally regulated directly or indirectly through TLR signaling [37-38]. Recently, it was showed that treatment of prediabetic mice with a synthetic TLR2 agonist diminished T1D and increased the number and function of CD4 and CD25 T cells, also conferring DCs with tolerogenic properties, suggesting that TLR2 signaling improves immunoregulation to prevent T1D [39]. On the other hand, another study suggested that TLR2 and MyD88 was dispensable for development of T1D in non obese diabetic (NOD) mice [40], which exhibit a susceptibility to spontaneous development of autoimmune insulin-dependent diabetes mellitus. NOD mice are a well established model of autoimmune diseases, including human T1D [41]. These data contrast with other reports showing the involvement of TLR2 in the initiation of autoimmune responses directed against beta-cells [42].

In NOD mice, the deletion of TLR4 results in acceleration of diabetes onset and immune cell infiltration of islets [43]. A recent study in the same animal model showed that TLR4 mediates cardiac lipid accumulation and diabetic heart disease [44]. On the contrary, treatment with a TLR4/MD-2 specific agonist monoclonal antibody (UT18) in NOD mice not only prevented T1D but also reversed new T1D onset diagnosed by polyuria, weight loss, and elevated blood glucose [45]. Supporting these results, an agonistic monoclonal antibody to TLR4/MD-2 reverses the development of diabetes in a high percentage of NOD mice. TLR4 antibody treatment increases T regulatory cell numbers in both the periphery and the pancreatic islet, suggesting a novel immunological tool for management of T1D in humans [46]. Taken together, these observations suggest a potential role for TLR2 and TLR4 in the pathology of diabetes. However, the mechanistic details need to be better investigated. Undoubtedly, the majority of existing data suggest that TLRs play a part in T1D, mediating T1D development and its complications, even if it is not clear if this is a beneficial role, a detrimental role, or a combination of the two.

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2.2. Other TLRs and T1D

The role of the TLR pathway in the mechanism of T1D has been intensely investigated in the last decade, and it is undeniable that these receptors are involved in the pathogenesis of T1D. However, the majority of the studies are focused on TLR2 and TLR4 and only a few studies have discussed other TLRs such as TLR1, TLR3, TLR7, and TLR9.

To the best of our knowledge, there is only one study showing that TLR1 may be involved in the mechanism of diabetes. In this work, a detailed phenotypic analysis of the diabetes-resistant NOD.C3H-congenic strain 6 was evaluated, and the results suggested that TLR1 pathway is involved in the inflammatory response and the development of T1D controlled by the Idd6 locus [47].

The TLR3 gene codes for an endoplasmic receptor that recognizes dsRNA and plays an important role in the innate immune response initiated by viral infection. Although there are only a few studies reporting a link between T1D and TLR3 gene alterations, polymorphisms in the TLR3 gene seem to be linked to the risk of T1D. In fact, rs5743313 and rs117221827 polymorphisms were associated with an early age at diagnosis and worse glycemic control [48]. However, genotypic data on a small population of South Africans of Zulu origin suggested a weak association of the TLR3 polymorphism C2593T, C2642A, and A2690G with T1D [49]. The hypothesis that viral infections are involved in T1D is based on epidemiological studies [50]. One of the major observations that support a role for a viral etiology of T1D is that in accordance rates for T1D in monozygotic twins are only 50% instead of the expected 100% if the characteristic would be explained only by genetic factors. TLR3 is expressed at high levels in human and mouse pancreatic beta-cells and antigen-presenting DCs, and this receptor activates the TRIF-mediated pathway, which in turn activates interferon regulatory factor (IRF)-3 inducing the expression of IFNs. However, NF-κB may also be activated by TLR3 to upregulate the production of proinflammatory cytokines [5].

TLR7 stimulation activates DCs and T cells to promote autoimmune diabetes in nonobese diabetic (NOD) mice. Treatment with IRS661, an antagonist for TLR7, inhibits the activation of DCs and CD8 T cells, as well as diminishes insulitis and diabetes onset in NOD mice [51]. Daily administration of a specific TLR7 ligand, 1V136, reduces autoimmune disease and modulates DC function [28]. In addition, treatment with 1Z1, an innate immune modulator generated by conjugating a TLR7 ligand to six units of polyethylene glycol (PEG), effectively prevented the clinical onset of hyperglycemia and reduced islet inflammation in NOD mice [52].

The involvement of TLR9 in T1D has been demonstrated in a rat model (diabetes-resistant BioBreeding or BBDR), which developed the disease following virus infection. In this study, disease progression was dependent on TLR9 signaling, leading to the activation of splenic B cells and bone marrow derived DCs [53]. A recent study investigating DCs subpopulations and their responses to TLRs stimulation in T1D patients, and their relatives showed increased TLR9-mediated interferon-alpha production in the first-degree relatives of T1D patients [27].

3. Contribution of TLRs to T1D complications

Diabetes leads to both microvascular and macrovascular complications. Many studies have shown increased levels of inflammatory biomarkers that could predispose to vascular complications. It is undeniable that TLRs are emerging as major factors in many diseases conditions owing to the activation of signaling pathways leading to the expression of inflammatory mediators and induction of immune responses.

Importantly, members of the TLR family play critical roles in the inflammatory components of vascular pathologies, including atherosclerosis [54-55], a condition characterized by inflammation of the vessel wall of the arterial tree. Atherosclerosis is an important vascular complication and the major cause of morbidity and mortality in diabetic patients [56]. Also, diabetes itself is a risk factor for atherosclerosis. Despite the fact that type 1 diabetics are at lower risk for atherosclerotic cardiovascular disease than type 2 diabetics because of the younger age of the former group, the relative risk is 10 times higher in type 1 diabetics than in nondiabetics of similar age [57]. Moreover, T1D has been linked with increased intima media thickness and impaired endothelial function (6), which affects vascular homeostasis leading to complications in diabetes. Lastly, hyperglycemia is a hallmark of diabetes and the role of glucose in the pathogenesis of atherosclerosis has been intensely discussed [58].

Devaraj et al. [30] reported on the role of TLR2 in the proinflammatory state in diabetes and incipient diabetic nephropathy. In TLR2 knockout streptozotocin (STZ)-induced diabetic animals, these authors observed a significant reduction in the NF-κB activity in peritoneal macrophages as well as in the release of various pro-inflammatory cytokines such as IL-6 and IL-8 compared to wild-type diabetic mice. Moreover, TLR2 KO STZ mice showed a significant decreased in albuminuria compared to WT-STZ, as well as increase in podocyte (epithelial cell in the kidneys) number, decrease in podocyte effacement, and a decrease in macrophages in the kidney. This study clearly implicates the TLR pathway in the genesis of a vascular complication in diabetes and demonstrates greater TLR activity in T1D.

Reactive oxygen species (ROS) formed in the vascular wall target a wide range of signaling molecules in both endothelium and vascular smooth muscle and contribute to vascular damage. Vascular dysfunction and remodeling through oxidative damage involves increased production and/or decreased degradation of ROS. One of the main enzymes implicated in vascular ROS generation is NADPH oxidase, although the mechanism behind induction of vascular NADPH oxidase activation in diabetes is less clear [59]. Recently, the role of TLRs in increased ROS levels in diabetes has been investigated [60-61]. It has been shown that the KO of the $P47^{phox}$ subunit of NADPH oxidase prevents diet-induced obesity via upregulation of both TLR2 and TLR4 [61]. A study addressing diabetic retinopathy using human retinal endothelial treated with high glucose showed that hyperglycemia induces TLR2 and TLR4 activation and downstream TLR signaling mediates augmented inflammation possibly via ROS [62], suggesting a mechanism by which TLRs could contribute to vascular damage in diabetes. However, the precise ligands involved in the activation of TLRs by hyperglycemia are still under investigation, and certainly this information will provide new insight for a role of TLRs in diabetes-associated vascular complications.

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4. Endogenous ligands (DAMPS) for TLRs in the mechanism of T1D

A large number of endogenous molecules may be potent activators of the innate immune system via TLRs leading to the release of proinflammatory cytokines from monocytes/macrophages. Unfortunately, there are limited data on the levels of endogenous ligands of TLR2 and TLR4 in T1D; however, a significant elevation of some ligands for TLR2 and TLR4 in T2D has been recently found. Overall, S100, fibrinogen, hyaluronan, oxidized LDL, and advanced glycation end products (AGE) showed increased levels in diabetic conditions and may work as DAMPS for TLRs [63]. However, high-mobility group box-1 protein (HMGB1), heat shock proteins (HSPs), and growth arrest-specific 6 protein (GAS6) are the ligands for TLRs specifically associated with T1D have been highlighted in the current literature.

4.1. HMGB1

HMGB1 was initially identified nearly 30 years ago as a chromatin associated protein that is important for transcriptional regulation. HMGB1 helps organize DNA and facilitates the binding of several regulatory protein complexes to DNA [64]. In addition to its role in transcriptional regulation, HMGB1 has been shown to activate proinflammatory responses following its release by necrotic or injured cells into the extracellular environment [65]. This protein may also be actively secreted by monocytes/macrophages. HMGB1 is implicated in the pathogenesis of a number of diseases associated with inflammation and tissue injury [66], and recently, many studies have suggested that HMGB1 acts as an inflammatory trigger in autoimmune diseases working as a DAMP [67]. Although the receptor for advanced glycation end products (RAGE) was the first HMGB1 receptor to be identified, this interaction alone could not justify all of the observed effects of HMGB1 [68]. Many relevant reports have shown that HMGB1 binds not only to RAGE, but also to TLRs [69]. The group of receptors that respond to HMGB1 is still expanding and includes cell membrane expressed TLR4 and TLR2 and endosomal TLR3, TLR7, and TLR9 [70].

To date, the main TLRs implicated in HMGB1 signaling are TLR2 and TLR4, although it is unknown if these receptors are acting independently or together. HMGB1 function is altered in diabetes, and the signaling systems triggered by this protein are not completely understood. The levels of TLRs and HMGB1 have been shown to be increased in patients with T1D [71], and HMGB1 is highly expressed in the cytoplasm of the islets in diabetic mice compared with nondiabetic controls. Furthermore, HMGB1 has been observed to increase in the cytoplasm of the islets during the progression of diabetes [72], and the augmented expression of this protein was observed in the retinas of diabetic patients with retinopathy [73].

HMGB1 polypeptide by itself has a weak proinflammatory activity, but it acquires proinflammatory activity through binding to proinflammatory mediators [74]. It is of interest to note that high glucose concentrations upregulate HMGB1, a ligand to TLR2 and TLR4 known to produce inflammation through NF-κB activation in human endothelial cells [69]. A recent study showed that while infusion of small amounts of glucose results in oxidative and inflammatory stress in patients with T1D, insulin infusion exerts an anti-inflammatory effect by suppression of TLRs and HMGB1 in mononuclear cells of T1D patients [75]. Moreover, it

has been suggested that the activation of TLR4 and RAGE by HMGB1 mediates injury and inflammation by the activation of NF-κB in response to hyperglycemia [68]. A study using NOD mice to address the significance of HMGB1 in the natural history of diabetes showed that HMGB1 interacts with TLR4 in isolated islets. By examining the effects of anti-TLR4 antibodies on HMGB1 cell surfacing binding, the authors suggested that TLR4 is the main receptor for HMGB1 on beta-cells and that HMGB1 may signal through TLR4 to selectively impair beta-cells during the progression of T1D [72]. Overall, a considerable body of evidence suggests that a complex set of mechanisms involving HMGB1, RAGE, and TLRs play a significant role in the development of chronic inflammation in diabetes [68].

On the other hand, many studies have shown that HMGB1 has angiogenic properties in promoting endothelial cell sprouting and migration under hypoxic and necrotic conditions [76-77]. There are data suggesting that release of HMGB1 in wounds initiates TLR4-dependent responses that contribute to neovascularization [78]. In addition, research on the transcriptional profiles of angiogenic endothelial cells has revealed HMGB1 as a promising angiogenic factor [79]. Conversely, a potential role for HMGB1 in atherosclerosis [80] is possible, shown by increased HMGB1 expression in atherosclerotic lesions compared with normal arteries. Under some circumstances, HMGB1 may act as a double-edged sword, but it appears that HMGB1 as a ligand for TLRs in T1D plays a detrimental action. Despite these apparent conflicting results, HMGB1 has a central role in mediating local and systemic responses to several stimuli, is involved in TLRs pathways, and may have therapeutic relevance in T1D.

4.2. HSPs

Heat shock proteins (HSPs) were originally identified as a set of proteins that are upregulated by increases in temperature [81]. These proteins were named for their apparent molecular weight, for example, HSP70 and HSP60, and have been shown to be among the most highly conserved proteins in the cell. Some HSPs, like HSP60s, are already abundant proteins that are further upregulated during stressful conditions. Other HSPs, like HSP70, have homologues that are constitutively highly expressed (sometimes referred to as HSC70s) and other homologues that are stress inducible. Most HSPs function as molecular chaperones that assist in the folding of newly synthesized, misfolded, or damaged proteins. Their requirement in protein folding helps to explain the important role of HSPs in both stress and nonstress conditions and the high degree of HSP conservation.

In addition to their primary role in intracellular protein folding, HSPs have been co-opted in a variety of other pathways. HSPs play an important role in cellular pathways involving cellular growth, division, and apoptosis. HSPs have also been implicated in various aspects of immune system modulation [82]. For example, HSPs have been shown to be involved in antigen presentation through their ability to bind and traffic proteins and peptides.

While their primary function is inside the cell, during stress, HSPs can be expressed on cell surfaces or secreted. One of their extracellular functions is the cross presentation of various antigens that they can bind. In addition, extracellular and purified HSP70 and HSP60 have been shown to bind to TLR2 and TLR4, which results in NF-κB activation in a MyD88 and CD14-dependent manner [83]. HSPs can activate immune cells, including B cells, NK cells,

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DCs, macrophages, and T cells. However, these results have been somewhat controversial as some HSP preparations have been shown to be contaminated with bacterial molecules, including LPS. Nevertheless, the abundance of HSPs, their high level of conservation, and their roles in cellular stress and inflammation may all help to explain why HSPs have been classified as important components of DAMP signals.

HSP60 and HSP70 have been implicated as key players in T1D. Both mouse models of T1D (NOD mice) and human patients have T cells that recognize and are activated by HSP60 and HSP70 [84-86]. These activated T cells then go to the pancreatic islets and recognize self HSP60 as an autoantigen. Extracellular HSP70 levels are positively correlated with insulin resistance *in vivo* and can cause β-cell dysfunction and death *in vitro* [87]. In mouse models, exogenous HSP60 and immunogenic peptides from HSP60 have been shown to alter the effects of T cells and prevent further β-cell destruction [88]. One peptide, in particular, DiaPep277, has shown promise in phase II clinical trials [89-90]. Phase III clinical trials using this peptide have been completed, but problems with the data analysis have surfaced and a new analysis of the clinical data is currently underway and expected to be completed soon [91-92]. HSP60 and HSP70 have also been implicated in complications of T1D, including atherosclerosis, indicating that they may have multiple roles in T1D [93-95].

4.3. GAS6

Growth arrest-specific 6 (GAS6) protein is another endogenous ligand of TLR2 and TLR4 that has been studied in experimental models of diabetic nephropathy [96], which is the most common cause of end-stage renal diseases, affecting 30% of T1D patients [97]. GAS6 and its receptor Axl play a key role in the development of glomerular hypertrophy, a hallmark of the early phase of nephropathy. In diabetic rats, it has been demonstrated that GAS6/Axl mediates glomerular hypertrophy during diabetes [96]. However, there is a paucity of information about the association of GAS6 and TLRs, and the involvement of GAS6 as a ligand for TLRs in T1D is unknown. Recently, analyzing the levels of ligands of TLR2 and TLR4 in mononuclear cells isolated from blood samples collected in patients with T1D, Deveraj and coworkers showed increased levels of HSP60 and HMGB1, but no significant difference in the levels of GAS6 between T1D diabetic group and their matched controls [71]. Therefore, further studies are necessary to clarify if there is a link between GAS6 and TLRs in the pathogenesis of T1D.

5. Targeting TLRs to manage T1D

Studies into the mechanisms behind disease progression have tended focus on identifying the important cell types and pathways involved in T1D. Definitely, TLR pathways are involved in T1D, making these receptors a tempting immune-based therapeutic intervention to handle T1D. TLR2 and TLR4 have a potential role in mediating inflammation and consequently, the complications associated with diabetes, mainly vascular damage, making them attractive targets. The deficiency of TLR4 as well as TLR2 is associated with reduced atherosclerosis and inflammatory state in diabetic mice [30, 98]. Treatment with a TLR4 antagonist was shown to

inhibit vascular inflammation and atherogenesis in STZ-induced ApoE$^{-/-}$ diabetic mice, as well as lower serum cholesterol and triglyceride levels in nondiabetic ApoE$^{-/-}$ mice [99]. It has been shown that TLR9$^{-/-}$ NOD mice present a delay in the onset of diabetes with decreased IFN-α production and decreased diabetogenic CD8 T cells in pancreatic lymph nodes. Moreover, the addition of a TLR9 antagonist oligodeoxynucleotide or chloroquine inhibited bone-marrow-derived DCs activation and CD8 T cells priming in response to CpG, an agonist of TLR9 [100].

Conversely, TLR agonists have been successfully used in NOD mice to delay T1D by inducing tolerogenic responses [39, 101]. The TLR2 agonist Pam3CSK4, when administered chronically in NOD mice, inhibits the development of T1D. Also, diabetogenic T cell priming of DCs was attenuated by chronic treatment with Pam3CSK4, suggesting DC tolerance [20]. Furthermore, the combination of TLR2 tolerization and inhibition of dipeptydil peptidase 4 (DPP4), which has been demonstrated to ameliorate STZ-induced diabetes by increasing beta-cell mass [102], can reverse early-onset diabetes in NOD mice [101].

Briefly, two contradictory possibilities have been considered regarding TLRs as a target to manage T1D. One is based on the belief that TLRs mediate T1D onset and its complications. Therefore, targeting these receptors using antibody treatment, pharmacological antagonist, or genetic approaches could minimize diabetes complications and decrease inflammation. The second possibility is based on the strategy of inhibiting T1D by tolerance mechanisms. There are scientific reports for both possibilities, making these receptors an attractive future option for treatment of T1D. However, the exact role of TLRs in the pathogenesis of T1D is not completely understood and the initial event of T1D is not revealed. Therefore, therapeutic approaches for T1D using TLR targeting remain a mere theoretical alternative.

6. Conclusion

The idea that the upregulation of TLR pathways, under some circumstances, leads to the induction of proinflammatory responses, and islet destruction is consistent with emerging data in animal models of T1D. In addition, a large body of evidence suggests increased TLR activity in diabetic patients, and these receptors have been suggested to be involved in the pathogenesis of diabetic vasculopathies. Therefore, therapeutic strategies to prevent TLR-mediated inflammation in T1D via modulation of either receptors or their DAMPs ligands can be a welcome addition to the available approaches to deal with T1D and diabetic vascular complications. However, targeting TLRs themselves using approaches such as antagonists could pose the risk of compromising host immunity. In addition, while some antigen-based immunotherapies targeting TLR ligands have proven to be protective against T1D development in animal models, these protocols might not be successfully adaptable to human diabetic patients at the time of diagnosis due to the nature of pathogenic and tolerogenic antigen selection in animal models and human individuals [103].

In fact, there exist number crucial questions regarding TLRs and T1D mechanisms that remain to be addressed. One of the major questions is do TLR pathways promote the initiation or

effector phase of diabetes, or both? A more complete understanding of how the innate immune system via TLRs can modulate autoimmune responses to beta-cell antigens, as well as the mechanisms by which these receptors are contributing to triggering and tuning T1D is crucial, not only because this information can lead to clear elucidation of the role of the innate immune system in TD1 but also because it may clarify whether TLRs can be used as an innovative clinical approach to manage or prevent this disease.

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