Toll-like receptors and diabetes: a therapeutic perspective

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ABSTRACT

Diabetes is a multifactorial metabolic disorder that leads to a number of complications. Diabetes is estimated to affect 36 million people in the U.S.A., and the prevalence of diagnosed and undiagnosed diabetes is at 9.3% and continues to rise. Evidence from experimental animal models as well as humans has indicated that systemic inflammation plays a role in the pathophysiological processes of diabetes and is facilitated by innate immune responses. TLRs (Toll-like receptors) are key innate immune receptors that recognize conserved PAMPs (pathogen-associated molecular patterns), induce inflammatory responses essential for host defences and initiate an adaptive immune response. Although TLR expression is increased in a plethora of inflammatory disorders, the effects of metabolic aberrations on TLRs and their role in diabetes and its complications is still emerging. In the present paper, we provide a systematic review on how TLRs play a detrimental role in the pathogenic processes [increased blood sugar, NEFAs (non-esterified ‘free’ fatty acids), cytokines and ROS (reactive oxygen species)] that manifest diabetes. Furthermore, we will highlight some of the therapeutic strategies targeted at decreasing TLRs to abrogate inflammation in diabetes that may eventually result in decreased complications.

INTRODUCTION

Diabetes affects more than 348 million people worldwide and around 36 million in the U.S.A. [1,2]. The prevalence of diabetes is increasing, with the lifetime risk estimated at 38.5% for women and 32.8% for men in the U.S.A. [3]. Over the past decade, the search for cellular mechanisms and molecular relationships in diabetes and its complications has revealed a close association between nutrient excess and derangements in mediators of immunity and inflammation. Potential pathogenic mechanisms in diabetes include hyperglycaemia, IR (insulin resistance), oxidative stress and inflammation that could culminate in the increased susceptibility to complications [4–7]. Recent insights into the activation of the innate immune system and inflammation via TLR (Toll-like receptor) activation in diabetes has lead to significant interest in the key signalling mechanisms as novel therapeutic targets for a range of inflammatory and immune diseases [8–11]. In the present review, we provide a broad overview of the links between diabetes and dysregulated innate immune responses, with a focus on targeting TLR signalling pathways and consequent inflammatory responses.

Key words: hyperglycaemia, inflammation, insulin resistance, oxidative stress, Toll-like receptor, Type 2 diabetes.

Abbreviations: AGE, advanced glycation end-product; AngII, angiotensin II; ARB, angiotensin receptor blocker; AT1R, AngII type 1 receptor; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HMGBl, high-mobility-group B1; HSP, heat-shock protein; IKK, inhibitory κB kinase; IL, interleukin; IR, insulin resistance; IRAK, IL-1-receptor-associated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; miRNA, microRNA; MUFA, mono-unsaturated fatty acid; MyD88, myeloid differentiation factor 88; NEFA, non-esterified fatty acid; NF-κB, nuclear factor κB; PKC, protein kinase C; PPAR-γ, peroxisome-proliferator-activated receptor-γ; PUFA, polyunsaturated fatty acid; RAGE, receptor for AGEs; ROS, reactive oxygen species; SFA, saturated fatty acid; siRNA, small interfering RNA; TLR, Toll-like receptor; TNF, tumour necrosis factor; TRIF, Toll/IL-1 receptor domain-containing adaptor protein inducing interferon β; TZD, thiazolidinedione.

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TLRs IN DIABETES

The recognition of microbial components by mammalian TLRs plays an important role in the activation of the innate immune response and subsequent pro-inflammatory reactions. TLRs also interact with ligands generated at sites of injury [12–14]. TLRs present on the cell surface recognize bacterial and fungal components, whereas intracellular TLRs recognize viral or microbial nucleic acids. In addition, TLRs also interact with endogenous ligands, such as oxLDL (oxidized LDL), HSPs (heat-shock proteins) 60 and 70, fibrinogen and fibronectin, which are also elevated in diabetes [15–20] (Figure 1). Thus different TLRs are amenable to targeting by different types of agents. Among the TLRs, TLR2 and TLR4 play a critical role in the pathogenesis of IR, inflammation and diabetes, in both experimental and clinical conditions [21–28], and thus are the main focus of the present review.

The interactions among increased glucose levels, elevated NEFAs [non-esterified (‘free’) fatty acids] and resultant pro-inflammatory cytokines in diabetes have clear implications for the immune system [29,30]. Studies in animal models as well as humans have suggested that diabetes might be associated with changes in the innate immune response [9]. Mohammad et al. [22] reported increased TLR2 and TLR4 expression in bone-marrow-derived macrophages of Type 1 diabetic NOD mice, correlating with increased NF-κB (nuclear factor κB) activation in response to endotoxins and increased pro-inflammatory cytokines. Furthermore, they showed increased levels of LPS (lipopolysaccharide)-induced iNOS (inducible NO synthase), IL-12p40 and TNF (tumour necrosis factor-α) in bone-marrow-derived macrophages from newly diabetic NOD mice compared with macrophages from BALB/c or pre-diabetic NOD mice. Kim et al. [23] using TLR2−/−/TLR4−/−-knockout mice and NOD mice have demonstrated that TLR2 senses β-cell death and contributes to the instigation of autoimmune diabetes. Recently, we have shown [21] increased TLR2 and TLR4 expression, intracellular signalling and TLR-mediated inflammation in monocytes with a significant correlation with HbA1c (glycated haemoglobin) levels in diabetic patients. Creely et al. [25] have shown increased TLR2 expression in adipose tissue from Type 2 diabetic patients with strong correlations with plasma endotoxin levels. In addition, Song et al. [26] reported increased TLR4 mRNA expression in differentiating adipose tissue of db/db mice. Furthermore, Davis et al. [35] have shown that the TLR4-deficient 10ScN mouse strain (which has a 74-kb deletion on chromosome 4 that prevents TLR4 expression) fed on a diet rich in saturated fat is protected from systemic inflammation. Taken together, these observations suggest a potential role for TLR2 and TLR4 in the pathology of diabetes. Furthermore, recent findings have shown increased TLR2/TLR4 expression, signalling, ligands and functional activation in diabetic subjects without and with complications [21,31]. In experiments using peripheral blood mononuclear cells and inhibitors of the MAPK (mitogen-activated protein kinase) or IKK (inhibitory κB kinase) pathways have shown that AP-1 (activator protein-1) and NF-κB are central regulators of inflammatory reactions [32], insulin response and glucose metabolism [33]. In addition, one of the receptors important for developing late diabetic complications, RAGE [receptor for AGEs (advanced glycation end-products)], has been shown to participate in the innate

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**Figure 1** Schematic depiction of TLR2 and TLR4 signalling in cells and corresponding ligands

LTA, lipoteichoic acid; Mal, adaptor-like protein; TBK1, TNF-receptor-associated factor-associated NF-κB activator-binding kinase 1; TICAM-2, Toll/IL-1 receptor-containing adaptor molecule-2; TIRAP, Toll/IL-1 receptor domain-containing adaptor protein; TRAF, TNF-receptor-associated factor.
immune response and to behave as a pattern recognition receptor [34]. This implies that factors regulating the innate immune response might be also involved in late diabetic complications.

**MOLECULAR MECHANISMS LINKING HIGH GLUCOSE, NEFAs, CYTOKINES AND ROS (REACTIVE OXYGEN SPECIES) WITH TLR-MEDIATED INNATE IMMUNE RESPONSES IN DIABETES**

**Hyperglycaemia**

Hyperglycaemia technically means high blood sugar levels and is considered to be the major cause of diabetic complications [2]. The DCCT (Diabetes Control and Complications Trial)/EDIC (Epidemiology of Diabetes Interventions and Complications) study and UKPDS (UK Prospective Diabetes Study) have clearly established the role of hyperglycaemia in diabetes [36,37]. Hyperglycaemia induces damage in tissues via: (i) increased flux of glucose and other sugars through the polyol pathway; (ii) increased formation of AGEs in the cells; (iii) increased expression of RAGEs and activating ligands in vascular cells; (iv) activation of PKC (protein kinase C) isoforms; and (v) overactivity of the hexosamine pathway [4,29,34,38-40]. The majority of the published literature suggests that all of the above five mechanisms are activated by a single upstream event, namely the overproduction of ROS [4]. High glucose has been shown to induce inflammatory cytokines and chemokines, and p38 MAPK and NF-κB activity in both clinical and experimental systems [34,38-40]. We have shown that a high-glucose dose time-dependently induces a marked increase in TLR2 and TLR4 mRNA and protein expression in human monocytes [29], consistent with those reported in macrophages of atherosclerotic lesions [41], human endothelial cells [42], smooth muscle cells of coronary arteries [43], dendritic cells [44], keratinocytes [45], pre-adipocytes [46], adipocytes [47] and pancreatic islets [55]. Our findings also showed enhanced MyD88 (myeloid differentiation factor 88)-dependent signalling, increased NF-κB transactivation and significant pro-inflammatory cytokine secretion [29]. Inhibition of TLR2 and TLR4 together in monocytes using siRNAs (small interfering RNAs) was additive, resulting in a significant decrease in NF-κB activity, suggesting that activation of both receptors is critical [29]. This mechanism involves PKC and NADPH oxidase activation, thus elaborating the steps that potentiate TLR2- and TLR4-activation-mediated inflammation observed in diabetes. However, the fundamental question of how high glucose activates TLR2 and TLR4 in monocytes and how this leads to increased inflammation needs to be determined. Dimerization is a critical event in the functional activation of TLRs [48]. Luciferase reporter assays (an indirect method to demonstrate dimerization) suggest that high glucose induces TLR2 and TLR6 heterodimerization, via NF-κB activation and cytokine production [29,48]. These results provide key evidence linking hyperglycaemia with the activation of innate immune receptors.

**NEFAs**

It is well established that patients with metabolic disorders, such as dyslipidaemia, obesity and diabetes, have elevated serum concentrations of fatty acids [49]. Studies have demonstrated a link between SFAs (saturated fatty acids), inflammation and IR [28,50,51]. Shi et al. [28] reported a reduction in lipid-induced NF-κB activation and IR in adipose tissue of TLR4-null mice. Studies by Schwartz et al. [30] and others [52–55] have indicated that increased concentrations of SFAs lead to the activation of TLR2 and TLR4 potentially inducing inflammation, with supporting proof-of-concept in vivo studies [55a–55c]. Interestingly, SFAs and MUFAs (mono-unsaturated fatty acids) differ significantly in their contribution to inflammation [48,51]. Thus it is generally thought that SFAs induce inflammation [48], whereas MUFAs increase insulin sensitivity in diabetic patients [48] and healthy subjects [56]. Adding to this interesting finding, we have recently shown increased TLR2 and TLR4 expression and activity in monocytes of Type 2 diabetic patients [21] and the enhancing effects of oleate, palmitate and stearate on TLR2 and TLR4 expression in high glucose. Although palmitate and stearate significantly amplified TLR expression, and NF-κB and inflammatory factors in high glucose, oleate had no effect [57] in monocytes. Our findings are in line with previous reports indicating that stearate and palmitate, but not oleate, increased TLR expression and cytokine production in islets [55]. In future studies, we will investigate whether the major n−3 PUFAs (polyunsaturated fatty acids) DHA [docosahexaenoic acid (C₂₂₆₃)] and EPA [eicosapentaenoic acid (C₂₀₅₃)] [50] ameliorate high-glucose- and palmitate-induced TLR-mediated inflammatory effects in vitro and in Type 2 diabetic patients. Interestingly, recent findings using mouse models were consistent with our in vitro observations [58]. Thus deficiency in TLR2 protects mice from high-fat-diet-induced obesity by regulating basal and insulin-induced glucose uptake in adipocytes [58]. The absence of TLR4 was associated with reduced IR in diet-induced obese mice [28], and neutralization of TLR2 with an antisense-oligonucleotide-attenuated IR in high-fat-diet-fed mice [65]. Several other studies have reported that TLR4 deficiency in mice improves insulin sensitivity and lowers inflammation in diet-induced obesity [59–62]. Nguyen et al. [63] have shown that NEFAs can cause activation of RAW264.7 cells primarily via the JNK (c-Jun N-terminal kinase) signalling cascade, and that TLR2 and TLR4 are upstream of JNK and help
to transduce NEFA pro-inflammatory signals. Tripathy et al. [49] have shown that infusion of NEFAs caused an increase in ROS generation, NF-κB p65-dependent activity and plasma inflammatory cytokine levels in healthy humans, linking NEFAs and an increase in NF-κB, a key step in the induction of inflammation. Our cell culture experiments provide mechanistic details in support of previous studies in humans demonstrating the innate immune (TLR2/TLR4), and oxidative (p47phox and ROS) and inflammatory effects (NF-κB) of high-fat and high-carbohydrate meals [66–68].

**Cytokines**

Hyperglycaemia and elevated NEFA levels induce inflammation, characterized by the increased expression of pro-inflammatory cytokines and activation of NF-κB linked with IR and diabetes [64]. We [21] and others [38,69,70] have shown that high-glucose treatment activates monocytes and induces an increase in TNFα, IL-1β and MCP (monocyte chemoattractant protein)-1 gene expression. Our findings showing increased levels of these cytokines in the monocytes under high glucose were similar to those observed in bovine retinal endothelial cells [26], LPS-activated human monocytes [71], human macrophages [72], human pancreatic islets [73] and human aortic endothelial cells [74]. We have also shown that this increase in cytokine levels initiates at the cell surface via TLR activation and signal propagation [29]. TLRs activate two types of downstream signalling pathways: MyD88-dependent and MyD88-independent pathways [75,76]. TLR2 primarily signals through the MyD88-dependent pathway to induce inflammation. Recently, we provided the first evidence, using the STZ (streptozotocin)-induced diabetes model, that TLR2 knockout results in a significant decrease in diabetes-induced inflammation independent of TLR4, as TLR4 levels and its non-MyD88-dependent signalling proteins [TRIF (Toll/IL-1 receptor domain-containing adaptor protein inducer of interferon β) and IRF-3 (interferon regulatory factor-1)] were unaltered [76a]. The decrease in inflammation, i.e. release of pro-inflammatory cytokines and chemokines, was associated with a significant reduction in NF-κB activity, MyD88 and phosphorylation of IRAK (IL-1-receptor-associated kinase)-1. Similar findings were found when TLR2 was knocked down using siRNA in cells under high-glucose conditions [29]. It is worth mentioning that TLR2 deficiency in the diabetic milieu has a profound effect on MyD88-dependent signalling, even with increased TLR4 expression and warrants further investigation. NEFAs also induces inflammation in skeletal muscle and liver through activation of NF-κB, resulting in the release of several pro-inflammatory and pro-atherogenic cytokines [52–54]. Thus elevated NEFA levels (due to obesity or to high-fat feeding) cause IR in skeletal muscle and liver, which contribute to the development of Type 2 diabetes and produces inflammation. For example, palmitate activates the transcription factor NF-κB and induces the expression and secretion of IL-6 in human myotubes and adipocyte cultures [54].

Several laboratories have shown that PKC-dependent signalling is involved in the activation of NADPH oxidase and superoxide anion production in a diabetic milieu [71,77,78]. We have shown previously [78] that high glucose activates monocytes and endothelial cells to produce ROS [78] and that IL-1β levels are increased under hyperglycaemic conditions, an increase that was mediated through increased ROS and activation of PKCα/β, leading to increased IL-1β secretion via NF-κB in monocytes. There are studies demonstrating the adverse inflammatory effects of high glucose, IR and oxidative stress mediated by persistent TLR activation [28,29]. In previous studies, increased monocyte superoxide release under high glucose has been shown to occur via activation of PKCα [29,69,78]. In addition, the differential regulation of TLR2 and TLR4 by PKCα/β under high glucose is in line with those observed in neutrophils and murine macrophages [79]. Furthermore, Thallus-Bonke et al. [80] have reported that activation of NADPH oxidase via PKCα is a key mechanism in diabetic renal disease. PKCα activation plays a critical role in the ER (endoplasmic reticulum)-stress-mediated cell death in cardiac myocytes and ischaemic hearts of rats [81]. Bey et al. [82] have shown that PKCδ plays a pivotal role in stimulating monocyte NADPH oxidase activity through its regulation of the phosphorylation and translocation of p47phox. Besides, ROS has been shown to regulate TLR4-mediated activation of NF-κB and IL-8 expression [83]. All of these studies strengthen the hypothesis that TLR2 and TLR4 are upstream of PKCα and PKCδ and this is associated with p47phox-dependent NADPH oxidase activity under high glucose and NEFAs.

**ROS**

Changes in the reduction–oxidation balance of tissues can lead to a pro-inflammatory state, typically seen in metabolic injury and diabetes. ROS include reactive products such as superoxide anions, H2O2 and hydroxyl radicals, which are formed as by-products of mitochondrial oxidative phosphorylation [84–86], activation of phagocyte NADPH oxidase and glycoxidation via interactions with RAGEs [84] in diabetes. Mounting evidence indicates that TLRs may be involved in this response [87]. The consequences of inflammation from oxidative stress injuries can lead to complications, death or disability [87]. Recent studies have shown that haemorrhagic shock through HMGB1 (high-mobility-group B1) activates the TLR4/MyD88/IRAK-4 signalling pathway with concomitant activation of p38 MAPK and Akt pathways to initiate phosphorylation of p47phox, and subsequent activation of NADPH oxidase [87].
Polymorphonucelar-cell-derived ROS from NADPH oxidase significantly increases TLR2 up-regulation in adipose tissue macrophages and endothelial cells in haemorrhage shock. Thus understanding the pathways leading to the initial activation of inflammatory pathways in metabolic stress is essential for devising strategies to limit the detrimental consequences of the inflammatory response to metabolic injury. High glucose/palmitate-induced ROS mediate insulin resistance [88,89]. ROS can also be produced during β-oxidation of fatty acids, especially as a by-product of peroxisomal acyl-CoA oxidase activity. Additionally, ROS can be produced by dedicated enzymes, such as NADPH oxidase [86], present in phagocytic cells, where ROS are an important part of cellular defence mechanisms [87].

**THERAPEUTIC MODULATION OF TLRs**

The studies described above suggest the possibility that TLR-mediated inflammation is key in the pathological mechanisms underlying diabetes and the consequent development of complications, and may be suppressed by anti-inflammatory treatments [90,91]. Besides, the ability of TLRs to initiate and propagate inflammation makes them attractive therapeutic targets [92–94]. However, as with many targets for anti-inflammatory agents, the approach is empirical. Given the available literature on TLRs in diabetes and its complications, we can be optimistic that targeting them will prove useful. Some of the strategies currently used for decreasing inflammation in diabetes include using statins, PPAR-γ (peroxisome-proliferator-activated receptor-γ) agonists (for example TZD (thiazolidinediones)), ARBs (angiotensin receptor blockers), phytochemicals and n–3 fatty acids [84]. All of these treatments exert their pleiotropic effects by inhibiting TLR-mediated inflammation, besides controlling diabetes co-morbidities, such as hyperglycaemia, dyslipidaemia and hypertension. In the following sections, we will focus on some of the potential therapeutic options mentioned.

**Statins**

Statins are chemically defined as a HMG-CoA (3-hydroxy-3-methylglutaryl CoA) reductase inhibitors and have been shown to effectively lower LDL (low-density lipoprotein)-cholesterol levels and reduce cardiovascular events in diabetic and non-diabetic patients. The pleiotropic beneficial effects of statins appear to be via anti-inflammatory actions and exceed their cholesterol-lowering effects. Statin treatment results in a reduction in NF-κB activity and a subsequent decrease in pro-inflammatory cytokines such TNF-α and IL-6 [95–97]. Furthermore, statins inhibit LPS-mediated activation of human peripheral mononuclear cells and endothelial cells [98]. Findings indicate that the anti-inflammatory effects of statins may involve TLRs [99,100], and it has been shown that statins inhibit TLR4 and TLR2 expression [101], with a concomitant decrease in TLR signalling and effector cytokine/chemokine release. Therefore statins, which target TLR-mediated signalling pathways in human peripheral mononuclear cells and endothelial cells, may be a good strategy in preventing the chronic inflammation seen in diabetes and associated CVD (cardiovascular disease). This hypothesis was supported further by results showing the inhibitory effects of simvastatin and atorvastatin on monocyte TLR4 expression in normolipaemic patients and patients with high cholesterol levels [102,103]. However, the mode of statin action on TLR expression is not clear and requires more studies.

**PPAR-γ agonists (TZDs)**

The pathophysiology initiating the development of inflammation remains poorly understood, in part due to the complexity of the interaction of multiple cells (monocytes, macrophages, T-cells, adipocytes and endothelial cells) and organ systems plus the diversity of intracellular perturbations within these systems that mediate the development of diabetes [104,105]. Increasing our understanding of this biology will require the combination of studying cross-talking signalling networks and the pleiotropic effects of known therapeutic drugs. PPAR-γ is a member of the nuclear hormone receptor superfamily that plays an important role in the regulation of inflammatory and immune reactions [106]. Their protective effects in inflammatory diseases may be correlated with the suppression of a key pro-inflammatory transcription factor, NF-κB [107], up-regulation of antioxidants [108], inhibition of pro-inflammatory mediators [109] and prevention of monocyte migration, adhesion and infiltration [110]. Whether these effects are operational in the immune system is not clear and, if present, whether these changes are linked to inflammation is also unclear. As mentioned above, TLR activation by glucose is linked with the inflammation seen in diabetes [29]. Therefore to understand the mechanisms of the anti-inflammatory effects of pioglitazone, we [109] and others [111,112] have investigated whether selective PPAR-γ agonists are effective inhibitors of TLR activation both in vitro and in vivo. Thus it may be reasonable to postulate that PPAR-γ agonists could negatively regulate inflammatory responses through TLR pathways and play protective effects in diabetic complications. However, more experimental and clinical studies are warranted in this regard.

**ARBs**

AngII (angiotensin II), in addition to stimulating vasoconstriction, also induces an increase in ROS and a
pro-inflammatory phenotype via AT\textsubscript{1}Rs (AngII type 1 receptors). ARBs are widely used as antihypertensive drugs and have been reported to possess anti-inflammatory effects. AngII, following engagement of the AT\textsubscript{1}R, promotes vasoconstriction, oxidative stress, inflammation and atherosclerosis [113–116]. ARBs, such as candesartan, prevent cerebrovascular events and also help reduce the progression of coronary heart disease [117–119]. Candesartan is widely used for the treatment of high blood pressure [120], the management of chronic heart failure [121] and diabetic nephropathy [122], to reverse endothelial dysfunction [123] and to attenuate oxidative stress [124]. Candesartan has been reported to have anti-atherosclerotic effects, such as reducing neointimal formation in rats [125] and diminishing vascular inflammation [126–128]. Given the anti-inflammatory effects of ARBs and the expression of TLRs in inflammatory conditions, such as diabetes, we have shown that candesartan decreases TLR2 and TLR4 protein level and mRNA expression and reduces NF-κB p65-dependent activation, with a concomitant reduction in key inflammatory mediator production in vitro [129]. Furthermore, administration of candesartan to mice resulted in significant reduction in TLR2 and TLR4 expression compared with vehicle control C57BL/6 mice [129]. These findings may have pathophysiological and clinical implications for patients with chronic inflammatory diseases, such as diabetes [25] and atherosclerosis [130], because these patients have enhanced TLR2 and TLR4 expression, leading to increased inflammation via the expression of inflammatory mediators such as IL-1β, IL-6, TNF-α and MCP-1. Although further investigation is needed to clarify the precise mechanisms by which candesartan inhibits TLR2 and TLR4 expression, we suggest that the documented pleiotropic anti-inflammatory effects of ARBs and the prevailing cross-talk among signalling pathways involving AT\textsubscript{1}Rs and TLRs in diabetes warrant further research, as hypertension in diabetic patients promotes early cardiovascular events.

**Phytochemicals**

Phytochemicals are chemical compounds that occur naturally in plants and may affect human health. Several lines of evidence suggest that diabetes-related pathologies can be prevented or improved by the intake of phytochemicals that can control inflammation. Inflammatory responses in activated cells are regulated by master regulators of inflammation such as NF-κB [79,84]. Moreover, TLR2 and TLR4 are reported to mediate inflammation in activated cells via NF-κB signalling [131]. Therefore targeting these inflammatory receptors using phytochemicals may be a useful strategy to prevent or ameliorate the development of diabetes and its related diseases. Several plant-derived components can modulate inflammatory responses via various mechanisms, some of which are dependent on TLRs, whereas others are TLR-independent by attenuating downstream NF-κB signalling [132,133]. In this section, we introduce the beneficial effects of anti-inflammatory plant derived components in diabetes-induced inflammatory responses and pathologies. Recent studies have shown that certain phytochemicals inhibit TLR-mediated pro-inflammatory. Capsaicin, the spicy component in chilli peppers, has anti-inflammatory properties in addition to its metabolic properties. It exerts its anti-inflammatory effects in macrophages by inhibiting MCP-1 and IL-6 secretion, and NF-κB inactivation [133,134]. Ginger (Zingiber officinale Roscoe) is widely used as a spice and herbal medicine. The active ingredient in ginger, 6-shogaol, has potent anti-inflammatory properties. Luteolin, a flavone that is present in medicinal plants and in some vegetables and spices, has been shown to possess antioxidant, anti-inflammatory and anti-allergy properties. Naringenin chalcone, a type of flavanoid present in tomato peel has been shown to have anti-allergic activities [135] and also suppresses the production of inflammatory mediators. However, it is not known whether the anti-inflammatory actions of capsaicin, ginger, luteolin and naringenin chalcone are mediated by TLRs.

Curcumin (turmeric), helenalin (a bitter component found in Arnica chamissonis) and cinnamaldehyde (cinnamon) have been shown to inhibit TLR4 activation by interfering with cysteine-residue-mediated receptor dimerization in HCT116 cells [132]. In contrast, resveratrol (a natural phenol found in red wine), EGCG (epigallocatechin gallate; a major component of green tea), luteolin (a flavonoid found in celery, green pepper and thyme) and the structural analogues of luteolin specifically inhibit TLR4 signalling by targeting TBK1 (TNF-receptor-associated factor-associated NF-κB activator-binding kinase 1) and RIP1 (receptor-interacting protein 1) in the TRIF complex in HEK (human embryonic kidney)-293 cells [132]. Together, these results suggest that TLRs and downstream signalling components are molecular targets for dietary strategies to reduce TLR-mediated chronic inflammation and the consequent risks of chronic diseases. However, most of these findings need to be validated using appropriate animal models and translation of the results to human studies, which is an active area of future investigations in diabetes and its complications. There are several unanswered questions with regards to the efficacy, mode of administration and amounts of the phytochemicals required for an optimal beneficial response with all of the different treatment options. Besides, it is also not know whether these treatment options help in preventing diabetic complications by inhibiting the different pathologies associated with diabetes. Furthermore, it is also unclear how different phytochemicals interact in vivo and this is a major area...
Figure 2 Schematic diagram showing areas of possible therapeutic intervention to regulate TLR2/TLR4 expression and activity in the pathogenesis of diabetes

The central hypothesis is to attenuate inflammatory processes and it is proposed that this would decrease susceptibility to diabetic complications. This schematic is generic and could be applied to most cell types.

for research investigation as they hold promise in limiting the inflammatory damage in diabetes.

**Omega-3 fatty acids**

As discussed above, SFAs directly induce inflammatory responses in macrophages via TLR2/TLR4, whereas long-chain \( n-3 \) PUFAs, such as DHA and EPA, are known to inhibit TLR2/TLR4 expression, activity and downstream signalling, and are considered as potential anti-inflammatory agents [49,50,132,136]. Fish oil contains high concentrations of DHA and EPA, and is considered to be a good source of \( n-3 \) PUFAs. TLR2/TLR4 activity and PPAR-\( \gamma \) activation is involved in the prevention of high-fat or high-energy-diet-induced tissue inflammation and remodelling by long-chain \( n-3 \) PUFAs [137,138], suggesting that the anti-inflammatory mechanisms of \( n-3 \) PUFA action are diverse and require detailed investigation [139]. Furthermore, \( n-3 \) PUFAs need many cofactors, such as folic acid, vitamins, tetrahydrobiopterin, minerals and \( L \)-arginine, for their physiological functions [140]. Hence these cofactors should also be provided in adequate amounts to bring about the anti-inflammatory actions of \( n-3 \) PUFA in obesity and diabetes. Further research on the effective amounts and forms of intake of \( n-3 \) PUFAs will help promote the development of PUFAs as potential anti-inflammatory therapy.

**THE FUTURE OF MODULATORS OF TLRs**

Increasing evidence using well-characterized approaches suggests the involvement of TLRs in metabolic disturbances and bridging immune responses to metabolic homoeostasis. However, efforts to identify modulators of TLR-dependent signalling and inflammation in diabetes and complications are significantly understudied. To date, there are no approved therapeutic agents targeting TLR2/TLR4 that have been shown to play a pivotal role in initiating and propagating persistent inflammation in diabetes. On the other hand, there are a variety of small-molecule inhibitors, compounds or antibodies under different developmental stages for allergy (TLR4, Pollinex Quatro; Allergy Therapeutics), pain management (TLR4, AV411; Avigen), autoimmunity, chronic inflammation and ischaemia/reperfusion injury (TLR2, OPN305; Opsona Therapeutics), and inflammatory bowel disease and rheumatoid arthritis (TLR2, OPN401; Opsona Therapeutics) targeting the TLRs [88]. The availability of TLR structures may now aid medicinal chemistry in the rational design of small-molecule agonists or antagonists. Non-traditional approaches to drug discovery, such as miRNAs (microRNAs), which regulate genes involved in immune responses, have been identified [141]. Some miRNAs are negative regulators of TLRs and signalling (for example, \( miR146a/b \)), acting as a brake on the pathway, whereas others act as activators [142,143]. Because miRNAs are tissue/cell- specific, fine-tune gene expression and are 22 nucleotides in length, they offer potential for drug therapies, as therapeutic administration of a single miRNA has an impact on the expression of many genes. However, clinically this may present both benefits and some off-target effects. Scientists are developing approaches to target miRNAs with the addition of antagonirs and locked nucleic acid inhibitors [142]. These approaches need to be applied to target TLR-regulated miRNAs in diabetes and its complications. In addition, there are several other promising therapeutic strategies, such as the thiamine derivative benfotiamine (transketolase activators), PARP [poly(ADP-ribose) polymerase] inhibitors and SOD (superoxide dismutase)/catalase mimetics, are under development and appear promising [78]. However, it is not clear whether any of these new therapeutic agents
inhibit TLR activity and thereby decrease inflammation. Another interesting facet of TLR signalling involves the host–microbial interaction, defined as a dynamic mutualistic relationship between the host and microbes pivotal to the maintenance of immune homeostasis.

Presumably, the interaction between microbiota and TLR2/TLR4 serves unique functions inherent to inflammatory disease [144] and remains an active area of investigation, as little, to no, information is available in the pathology of diabetes.

In summary, as more details on TLR signalling in diabetes and its complications continue to emerge, it is imperative to develop and evaluate novel TLR modulators in pre-clinical and clinical models. It is clear that there are a number of new opportunities to target innate immune signalling. The recent illustration of miRNAs present an exciting avenue for drug discovery and development [141–143]. Both experimental and clinical results support our belief that the ability to target key receptors or signalling in innate immunity might prevent uncontrolled inflammation and limit the progression of many diseases, including diabetes [94,132] (Figure 2).

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