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Mini-Review

Toll-like receptor 2/4 links to free fatty acid-induced inflammation and β-cell dysfunction

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ABSTRACT

T2D is a metabolic and inflammatory disease characterized by deteriorating β-cell function and increased levels of inflammatory cytokines. Low-grade inflammation and innate immune system activation lead to β-cell failure. Recently, SFAs have been proposed as triggers of metabolism-associated inflammation through the TLR family of PRRs. In this review, recent progress in defining the molecular basis of FFA-associated TLR2/4 activation and signaling in β-cell dysfunction and apoptosis is summarized. Furthermore, we highlight links between TLRs and diabetic complications, insulin resistance, and autophagy. This knowledge may facilitate novel strategies to abrogate inflammation in T2D. *J. Leukoc. Biol.* 95: 47–52; 2014.

Introduction

T2D develops as a result of a combination of insulin resistance and pancreatic β-cell failure. Insulin resistance and islet β-cell dysfunction symptoms are believed to be caused by oxidative stress, ER stress, amyloid deposition in the pancreas, lipotoxicity, and glucotoxicity, all associated with excess nutrition or overeating. These cellular stresses are thought to induce an inflammatory response and be exacerbated by inflammation or associated with this pathophysiological state. Inflammation in pancreatic islets was confirmed recently in Zucker fa/fa rats, a model of obesity-associated insulin resistance [1]. Proinflammatory signaling pathways can inhibit insulin signaling [2]. Inflammatory cytokines converged on inhibitors of NF-κB, IKKβ, and MAPK/JNK1 to directly inhibit insulin action via serine phosphorylation of IRS1 and IRS2 [3]. Metabolic activation of the innate immune system governed by IL-1β contributed to β-cell failure in T2D [4]. The increased concentrations of IL-1β + IFN-γ and ER stress converged on Bcl-2 homology 3-only member death protein 5/hara-kiri in β-cell apoptosis [5]. In response to environmental threats, sentinel cells, such as macrophages, endothelial cells, and adipocytes, release inflammatory cytokines that stimulate the production of APPs, such as CRP and serum amyloid A. Increased CRP and cytokines (IL-1β, IL-6, and TNF-α) and chemokines (MCP-1, IL-8), observed in in vitro studies, could lead directly to impaired β-cell function and survival [6]. Elevated levels of APPs may predispose cells toward T2D, and increased IL-6 and CRP are closely correlated with the development of T2D [7]. Indeed, early experiments suggested that the anti-inflammatory effects of salicylates had an ameliorating effect on T2D [8], and an IL-1R antagonist improved blood glucose levels and β-cell function in T2D patients [9], providing a link among inflammation, β-cell dysfunction, and T2D.

TLRs, one family of PRRs, detect microbial components and induce the production of inflammatory cytokines by macrophages/DCs [10]. TLRs recognize a wide range of pathogen-associated molecular forms, including lipids, lipoproteins, and proteins [10], as well as endogenous ligands, including oxidized LDL, heat shock proteins 60 and 70, fibrinogen, and fibronectin, which are also elevated in diabetes [11–14]. There are at least 13 TLRs identified in mice and 11 in humans. TLR1–6 and -11 are membrane proteins, whereas TLR3 and -7–9 are present in intracellular compartments and are expressed on a range of cell types, including macrophages, DCs, endothelial and epithelial cells, and pancreatic islets [15, 16]. Increased, circulating TLR2 ligands were identified recently in T2D patients [17]. High glucose increased TLR2 and TLR4 expression and activity in human monocytes, stimulating MyD88-dependent signaling and culminating in NF-κB transactivation, leading to significant proinflammatory cytokine secretion [18]. TLR4 appeared to be involved directly in the pathophysiology of T2D [19] and played a direct role in β-cell dysfunction, as LPS impairs insulin gene expression via TLR4 and NF-κB signaling [19, 20].

Abbreviations: $^{-/-}$=knockout/deficicent, ApoE=apolipoprotein E, APP=acute-phase protein, CRP=C-reactive protein, FetA=fetuin-A, FFA=free fatty acid, HFD=high-fat diet, Ins2=mutant insulin 2 protein, IRAK=IL-1R-associated kinase, IRS=insulin receptor substrate, Pdx1=pancreatic-duodenal homeobox 1, premRNA=precursor mRNA, SFA=saturated free fatty acid, T2D=type 2 diabetes, TRIF=Toll-IL-1R domain-containing adaptor-inducing IFN

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Given the importance of TLRs in mediating inflammation in diabetes, it is conceivable that they may play a role in β-cell function and homeostasis. In this review, the involvement of TLR2/4 in β-cell inflammation, dysfunction, and FFA-induced apoptosis is summarized. However, fundamental questions need answers before TLRs can be considered a therapeutic target. We highlight some of the proposed therapeutic strategies associated with TLRs, which may provide novel approaches to abrogate inflammation in T2D.

FFA-INDUCED INFLAMMATION VIA TLR2/4 PATHWAYS

The interaction among increased glucose levels, elevated FFAs, and proinflammatory cytokines in diabetes has clear implications for the immune system [18, 21]. TLRs were able to sense pathological levels of lipid, and FFAs are detected through TLR2 and TLR4 in immune cells [22]. Obesity-associated inflammation is mediated, at least in part, by the effects of SFAs that stimulate intracellular proinflammatory pathways in a TLR4-dependent manner. TLR4 expressed in β cells [23] binds to CXCL10 to induce β-cell death by switching Akt signaling from proliferation to apoptosis [24]. TLR4 and NF-κB signaling were required for LPS impairment of β-cell gene expression [20]. The expression levels of genes, such as Pdx1, Ins2 premRNA, and insulin, could not be suppressed by palmitate in TLR4 knockdown β cells, also lacking the downstream molecule MyD88 [25]. The lack of MyD88 increased susceptibility to streptozotocin-induced apoptosis and caused a decrease in β-cell mass [26]. Hutton et al. [27] showed that deficiency in TRIF (a downstream molecule of TLR3) improved glucose tolerance and β-cell dysfunction. These results demonstrated that TLR signaling might also be important in the generation and/or replication of β cells.

TLR2/4 SIGNALING IN FFA-INDUCED β-CELL DYSFUNCTION AND APOPTOSIS

There are two major downstream signaling inflammatory pathways of TLRs; MyD88-dependent and independent pathways. These are differentially activated by each TLR agonist and lead to differential target gene expression and cellular responses. TLR2 and -9 induce NF-κB activation and cytokine production through the MyD88-dependent signaling pathway, whereas TLR4 and FFAs trigger the activation of both pathways [28].

MyD88 is an essential adapter protein for TLR/IL-1R signaling in metabolic disease [29]. MyD88 was identified originally as a myeloid differentiation primary response gene, which is induced in M1 myeloid leukemia cells in response to IL-6 [30]. Subsequently, MyD88 was found to be related to the IL-1R family, including Toll/TLR protein, which is homologous to that of IL-1R [31]. Finally, it was demonstrated that signaling via TLR4 uses MyD88 as an adaptor protein that induced activation of NF-κB through IRAK and TRAF6 [32]. The activated NF-κB was translocated to the nucleus, and this induced the expression of inflammatory cytokines. In the MyD88-independent pathway, Toll-IL-1R activation led to phosphorylation and nuclear translocation of IFN regulatory factor 3 and expression of IFN-β and IFN-inducible genes [33]. TLR2 was known to activate NF-κB through MyD88-dependent signaling pathways involving IRAK-1, TRAF6, and IKK [34]. The activation of TLR2 could also lead to the activation of PI-3K/Akt, resulting in enhanced transactivation of NF-κB [35]. FFAs activated TLR2, leading to the activation of MyD88/IRAK1/TRAF6/IKKβ and MyD88/PI-3K/AKT pathways [28]. In addition, dimerization of TLR2 with TLR6 or TLR1 on monocytes was induced by high glucose, and this may cause TLR2 activation, NF-κB activation, and production of cytokines.

Palmitate, one of the most abundant FFAs in blood, induced the accumulation of proinflammatory monocytes and macrophages within the islet, contributing to β-cell dysfunction. Such an accumulation could not be induced in the islet of $TLR4^{-/-}$ and $MyD88^{-/-}$ mice, indicating that the TLR4/MyD88 pathway is involved [25]. SFAs do not activate TLR1–6 or TLR9 in 293T cells. SFAs activate MyD88-independent signaling pathways through TLR4 but not TLR2. Activation of TLR2 by SFAs is indirect via dimerization with TLR1 and TLR6 [28]. In addition, β cells and macrophages interacted reciprocally, at least in part, via cytokines, to further augment and propagate inflammatory processes within the islets [25]. Depleting macrophages abolished the palmitate-induced down-regulation of Pdx1, insulin, and Ins2 premRNA and inhibited the up-regulation of IL-1 and TNF-α within islets [25].

TLR pathway-associated islet inflammation and β-cell dysfunction may involve several steps (**Fig. 1**): (1) β-cell response to endogenous stimuli, such as FFAs by activating TLR4/TLR2; (2) inflammatory cytokines are produced via the TLR4, MyD88-dependent/independent, TLR2-associated MyD88-dependent, MyD88/IRAK1/TRAF6/IKKβ, or MyD88/PI-3K/Akt

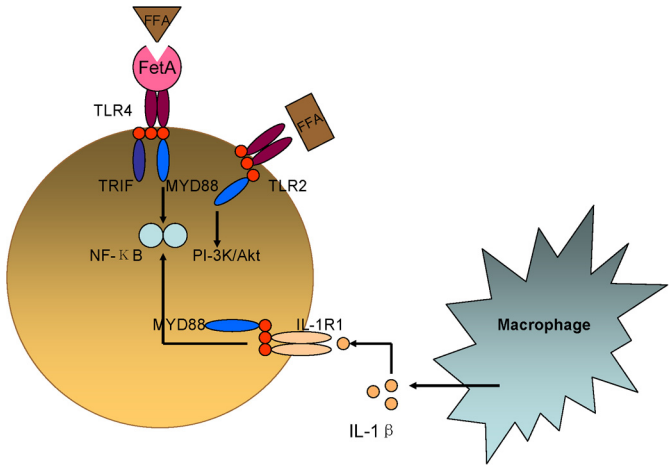

Figure 1. FFA-induced TLR2/4 signaling in β-cell inflammation. FFA induces the activation of TLR4 through FetA and causes β cells to produce inflammatory cytokines via the TLR4, MyD88-dependent and independent, TLR2, MyD88/IRAK1/TRAF6/IKKβ, and MyD88/PI-3K/Akt pathways. Inflammatory cytokines recruit macrophages that interact with β cells to perpetuate inflammatory processes that lead to β-cell dysfunction.

pathways; (3) inflammatory cytokines recruit macrophages that perpetuate inflammatory processes within the islets, eventually resulting in β-cell dysfunction.

Interestingly, SFAs and mono-unsaturated fatty acids differed significantly in their contribution to inflammation; in high glucose conditions, palmitate and stearate significantly amplified TLR expression, NF-κB, and inflammatory factors, whereas oleate had no effect on monocytes [36]. Whether the major n-3 polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid ameliorate high glucose- and palmitate-induced, TLR-mediated inflammatory effects in vivo and in T2D patients still needs addressing.

TLR2/4 PLAY PIVOTAL ROLES IN FFA-INDUCED INSULIN RESISTANCE

FFAs caused inflammatory responses in insulin-sensitive tissues, such as skeletal muscle and adipocytes, resulting in local and systemic insulin resistance. FFAs could stimulate macrophage and adipocyte inflammation by activating the NF-κB and JNK signaling pathways via TLR4 [37]. Pal et al. [38] showed that FetA, a liver-derived circulating glycoprotein, serves as an adaptor protein that directly links SFAs to TLR4 activation. Most convincingly, in vivo SFA infusion led to insulin resistance in control mice, whereas FetA-knockdown mice were protected from these effects. This hints at the exciting possibility that targeting the SFA-FetA-induced branch of TLR-mediated inflammation could have beneficial effects on improving glucose homeostasis in T2D without affecting immune function.

The pathophysiological importance of TLR4 in obesity-induced inflammation and insulin resistance was investigated in $TLR4^{-/-}$ mice signaling as a result of direct gene knockout ($TLR4^{-/-}$ mice) or a loss-of-function mutation in the TLR4 gene (C3H/HeJ and C57BL/10ScN) [37]. $TLR4^{-/-}$ overcame impaired insulin signaling and insulin resistance caused by lipid infusion in muscle [37], and $TLR4^{-/-}$ mice were protected against diet-induced insulin resistance [39]. It has been reported that the TLR4 status correlates with energy homeostasis, weight gain, and fat mass, exaggerated by HFD [40]. This phenotype could be related to a protection against diet-induced leptin or insulin resistance in the hypothalamus in the absence of functional TLR4 signaling [41]. However, myeloid cell-specific TLR4 deletion protected cells against diet-induced obesity and insulin resistance, indicating that macrophages are the main mediator of the TLR4 response in adipose tissues [42].

As with TLR4, $TLR2^{-/-}$ also improved diet-induced insulin resistance and inflammation of adipose tissue, liver, or muscles [43–45]. Furthermore, RNA interference-mediated inhibition of TLR2 in cultured muscle cells resulted in a near-complete inhibition of palmitate-induced insulin resistance [46]. TLR4 and TLR2 therefore appear to be required components for FFA-induced insulin resistance in adipocytes and other cells that result from HFDs. This suggests a previously unreported link between the innate immune system and metabolism.

TLR2/4 AND DIABETES-ASSOCIATED COMPLICATIONS

T2D confers an increased risk of macrovascular complications, as shown by the increased levels of inflammation biomarkers [47]. Receptor for advanced glycation end product is involved in the development of late diabetic complications, participates in the innate immune response, and acts as a PRR [48]. Increased TLR2/TLR4 expression, signaling, ligands, and functional activation have been shown in diabetes subjects with and without complications [17, 49]. Vascular inflammation and impaired insulin responsiveness were compared in aortic samples obtained from WT and $TLR4^{-/-}$ mice fed a HFD. The $TLR4^{-/-}$ mice were protected against HFD-induced vascular inflammation [50]. TLR4 has also been shown to contribute to early-stage intimal foam-cell accumulation at lesion-prone aortic sites in $ApoE^{-/-}$ mice, as has TLR2 to a lesser extent [51]. $TLR4^{-/-}$ reduced intimal lipid by ~75% in $ApoE^{-/-}$ mice, whereas $TLR2^{-/-}$ reduced it by 45%. Excessive accumulation of lipids in macrophages, resulting in foam-cell formation, is a hallmark of atherosclerosis. Resistin, originally described as an adipose tissue-specific hormone, was found to be involved in pathological processes leading to cardiovascular disease, such as inflammation, endothelial dysfunction, thrombosis, angiogenesis, and smooth muscle cell dysfunction. TLR4 served as a receptor for the proinflammatory effects of resistin in human cells [37, 52]. This may partly explain the multifunctional role of resistin in chronic inflammation, atherosclerosis, and insulin resistance. TLR2 expression was increased in endothelial cells at sites prone to atherosclerosis [53]. $ApoE^{-/-}$ mice, deficient in TLR4 and TLR2, displayed a 55% decrease in atherosclerotic lesion development, whereas a 65% decrease in macrophage infiltration was seen in $ApoE^{-/-}$ mice deficient in TLR4 alone [54, 55]. These results suggest that diabetic complications are associated, in part, with the release of endogenous TLR ligands that lead to activation of TLR signaling. TLRs are therefore of primary importance in initiating and mediating the immune response and may be attractive targets for eliminating or minimizing diabetic complications.

TLRs AND AUTOPHAGY

Autophagy is a highly evolutionarily conserved process that can occur in almost all eukaryotic cells. It involves the sequestration of regions of the cytosol within double-membrane-bound compartments and delivery of the contents to the lysosome for degradation. In samples from T2D patients, increased amounts of dead β cells with signs of altered autophagy have been identified [56]. Autophagy is involved in insulin resistance and ER stress-induced β-cell death [57]. Inhibiting autophagosome formation augmented FFA-induced β-cell death [58]. The role of autophagy as a pro-survival or -death mechanism is a highly controversial subject [59]. Evidence suggests that basal autophagy has anti-inflammatory effects by suppressing unscheduled inflammasome activation [60–62], whereas induced autophagy promotes inflammasome secretion of cytokines such as IL-1β [63].

TLRs were historically the first class of PRRs to be connected with autophagy [64]. LPS-induced autophagy was observed to be markedly inhibited in cells transfected with a vector that overproduces an inactive TLR4, and knockdown of TLR4 using small interfering RNA completely abrogated the induction of autophagy by LPS [65]. Delgado et al. [66] showed that TLR7 ligands were a potent inducer of autophagy, via MyD88 as a downstream adaptor, and this could eliminate intracellular pathogens. These studies provide evidence for a connection between TLR signaling and autophagy and hint at the possibility of inducing selective autophagy to protect against FFA-induced β-cell dysfunction.

THERAPEUTIC POTENTIAL OF TARGETING TLRs

Anti-inflammatory approaches have been used in the treatment of T2D and insulin resistance [67]. Results from a randomized, placebo-controlled trial of an anti-IL-1β antibody showed an improved insulin secretion rate, supporting the hypothesis that insulin secretion can be improved by blocking IL-1β [67]. Gevokizumab, a novel-engineered anti-IL-1β mAb, improved glycemia and reduced inflammation in patients with T2D, possibly via the restoration of insulin production and action [4]. The blockage of TLRs and their pathways could provide novel therapeutic strategies for the treatment of T2D.

TLR4$^{-/-}$ ameliorated impaired insulin signaling in muscle and reduced insulin-mediated glucose metabolism induced by lipid infusion [68]. TLR4 pathways can conceivably be blocked in a variety of ways. The adipocytokine visfatin was shown to be involved in the regulation of inflammation and apoptosis through activation of MAPK- and PI3K-dependent signaling pathways [69], which converge with the TLR-associated MyD88/PI-3K/AKT pathway, providing a potential intervention point for TLR-related therapies.

Cha et al. [70] have reported the renal, protective effects of blockading TLR4 signaling in T2D mice. In cultured podocytes and adipocytes, high levels of glucose and FFAs stimulated TLR4 expression and production of proinflammatory cytokines, but the effects were abolished by inhibiting TLR4- and TLR2/6-mediated signaling pathways. In another study, the synthetic lipid Eritoran was found to bind to the myeloid differentiation protein 2 adaptor protein, preventing LPS activation of TLR4, and is therefore useful in the treatment of inflammatory diseases [71]. Eritoran is structurally similar to the LPS lipid A structure and presumably functions as a TLR4 antagonist [71].

Specific mAb represent another way of inhibiting the activation of TLRs. To this end, the anti-hTLR4-IgG antibody (InvivoGen, San Diego, CA, USA) has been developed, which may function by neutralizing human TLR4-induced cellular activation [72]. The anesthetic ketamine reduced the release of proinflammatory cytokines and decreased TLR4 expression [73], indicating the potential of this or similar compounds for the treatment of chronic inflammatory processes and metabolic diseases, such as obesity and T2D. All of the TLRs except TLR3 can act via the MyD88-dependent pathway. Therefore, it was possible that deactivation of MyD88 may attenuate the TLR-mediated cytokine pathway. High doses of cinnamon extract were found to suppress the induced overexpression of MyD88 in vitro and in vivo [74].

Macrophages can be induced through the combined action of TLRs and other immune system stimuli. Manipulating macrophage migration inhibitory factor may be therapeutically beneficial for the treatment and prevention of obesity-related metabolic disease. TLR4 serves as an environmental sensor for autophagy [75]. A new molecular pathway was defined in which LPS-induced autophagy was regulated through TRIF-dependent, MyD88-independent TLR4 signaling [75]. Therefore, control of autophagy (discussed above) presents a potential, novel therapeutic strategy for T2D and other metabolic diseases.

CONCLUSIONS

TLRs and inflammation are critical components in the pathology of β-cell dysfunction and cell death in T2D. This review brings together recent developments that have revealed a link between TLR2/4 and FFA-induced inflammation and autophagy that impacts directly on T2D and complications associated with the disease. Evidence for the role of inflammation in metabolic diseases, such as T2D and obesity, has also been collated. FFA-induced, TLR2/4-associated downstream signaling in β cells is discussed, and potential therapeutic strategies involving targeting of TLR2/4 are detailed. The field is ripe for further work in these areas.

In summary, (1) a strong link between TLRs and inflammation has been established, but the molecular mechanisms and their role in T2D patients require further studies; (2) macrophage activation plays an important role in inflammation leading to diabetic complications; the targeting of TLR2/4 in macrophages has potential as a novel therapeutic strategy in metabolic diseases, such as T2D; and (3) the link between TLRs and autophagy is interesting, and further studies in this area may lead to a better understanding of the role of inflammation in β-cell dysfunction.

AUTHORSHIP

J.Y. is the first author. Y.P. is the corresponding author. J.W., Y.W., and L.Y. contributed equally.

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DISCLOSURES

The authors declare no conflict of interest.

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