A Novel Small Molecule Drug Derived from Methimazole (Phenylmethimazole) that Targets Aberrant Toll-like Receptor Expression and Signaling for the Potential Prevention or Treatment of Diabetes Mellitus and Non-alcoholic Fatty Liver Disease

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Abstract

Toll-like receptors (TLRs) are pattern-recognition receptors located on the surface or within (endosome) immune cells (dendritic), whose function is to recognize pathogens from the environment and mediate both the innate and acquired immune responses. Environmental activation of TLRs in nonimmune cells is now recognized as a significant pathway that mediates the loss of self-tolerance in autoimmune diseases, as well as inflammation-induced cell damage in many chronic diseases. We have developed a small molecule drug derived from methimazole, phenylmethimazole (C10), which interferes with the environmental induction of TLR signaling in nonimmune cells and is an active inhibitor of pathologic inflammation in many animal disease models. This article has been written to introduce clinicians to TLR function and the potential therapeutic role that their inhibition could play in many inflammatory/autoimmune diseases, including diabetes and nonalcoholic fatty liver disease (NAFLD).

Keywords

Toll-like receptors, diabetes, nonalcoholic fatty liver disease, inflammation, TLR antagonists

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There are two major forms of diabetes mellitus, type 1 (T1DM) and type 2 (T2DM), with the latter being the most common form, accounting for over 90 % of cases.1 Further, with the epidemic of obesity, T2DM can occur at any age, including children and adolescents,1 affecting nearly 30 million in the US and 400 million worldwide.2 Although there are multiple medications available to treat T2DM, overall success is poor given that no medication addresses the multiple abnormalities associated with T2DM and changes in diet and lifestyle are extremely difficult to achieve.2 T1DM occurs more frequently in children, adolescents, and young adults and is caused by destruction of the insulin-producing beta cells of the pancreas.3,4 Currently there is no cure for T1DM and it requires lifelong insulin replacement.4 The prevalence of both forms of DM are increasing worldwide3,4 and are associated with increased risk for multiple long-term diabetes complications, premature disability, and early death.2,4,5 Nonalcoholic fatty liver disease (NAFLD) is even more common than T2DM affecting 75 % of all obese individuals, is the leading cause of cirrhosis, end-stage liver disease, and primary hepatocellular cancer in the US.1,6 Treatment of NAFLD is also difficult due to similar lifestyle change requirements,7 and many of the medications used to reduce hepatic inflammation and fibrosis are potentially hepatotoxic themselves.8,9,10 Thus, all of these diseases (as well as atherosclerosis and many cancers) have a large inflammatory/immune component, which is driven by environmental insults to the body (viruses, dietary components such as high saturated fats, or overconsumption of high-fructose corn syrup [HFCS], etc.) Much contemporary research is directed at suppressing the various inflammatory pathways involved in these diseases without impairing the normal immune response or damaging the tissues we are attempting to protect.

Toll-like receptors (TLRs) are pattern-recognition receptors that were originally identified on the surface or within (endosome) immune cells (dendritic and monocytes/macrophages) that recognize various pathogen-associated molecular patterns (PAMPs) from the environment.11,12 Recognition of these PAMPs by the various TLRs protect mammals from pathogenic organisms, such as bacteria, viruses, or protozoa and are important mediators of both the initial “innate” immune response to the molecular products of these organisms as well as the “adaptive” or memory immune response.13 TLRs
were consequently found to be expressed in multiple nonimmune cell types including the epithelial cells lining the gastrointestinal tract,\textsuperscript{36} endothelium,\textsuperscript{20} liver,\textsuperscript{21,22} adipocytes,\textsuperscript{23,24} and even pancreatic beta cells.\textsuperscript{25} TLR also recognize endogenous ligands released by injured cells (from infection, inflammation, radiation, etc.), and these are termed damage-associated molecular patterns (DAMPs). Ten different TLRs have now been identified in humans, as well as many of their exogenous and/or endogenous ligands (except TLR 10 whose ligands are not yet known), as well as some of the diseases to which they are linked (see Table 1).

We first became interested in TLR function when attempting to determine the molecular mechanism of action of the drug phenylmethimazole (C10), which is a derivative of the antithyroid medication methimazole. Methimazole is commonly used to treat hyperthyroidism by blocking thyroxine biosynthesis in thyrocytes; however, methimazole was also shown to downregulate expression of the major histocompatibility (MHC) genes, I and II,\textsuperscript{26,27} which suggested it might also possess anti-inflammatory and immunoregulatory activity. C10 (which does not have any effect on thyroxine biosynthesis) was suggested it might also possess anti-inflammatory and immunoregulatory activity. C10 (which does not have any effect on thyroxine biosynthesis) was consequently found to be expressed in multiple nonimmune cell types including the epithelial cells lining the gastrointestinal tract,\textsuperscript{36} endothelium,\textsuperscript{20} liver,\textsuperscript{21,22} adipocytes,\textsuperscript{23,24} and even pancreatic beta cells.\textsuperscript{25} TLR also recognize endogenous ligands released by injured cells (from infection, inflammation, radiation, etc.), and these are termed damage-associated molecular patterns (DAMPs). Ten different TLRs have now been identified in humans, as well as many of their exogenous and/or endogenous ligands (except TLR 10 whose ligands are not yet known), as well as some of the diseases to which they are linked (see Table 1).

Table 1: Toll-like Receptors, their Location, Specific Ligands, and Disease Associations

<table>
<thead>
<tr>
<th>Human Toll-like Receptor</th>
<th>PAMPs/DAMPs/Other Ligands</th>
<th>Disease Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Glycolipids, triacyl lipopeptides, heat shock proteins</td>
<td>Cancer, psoriasis, sepsis, leprosy</td>
</tr>
<tr>
<td>TLR2</td>
<td>Lipo-polyaccharides, saturated free fatty acids</td>
<td>Cancer, type 2 diabetes, psoriasis, sepsis, leprosy, filariasis, asthma, arthritis</td>
</tr>
<tr>
<td>TLR3</td>
<td>Poly(C)</td>
<td>Cancer, type 1 diabetes, leprosy</td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipopolysaccharides, saturated free fatty acids</td>
<td>Cancer, type 2 diabetes, psoriasis, sepsis, leprosy, rheumatoid arthritis, chronic obstructive pulmonary disease, diabetic nephropathy, subnephrotic dermatis</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Cancer, psoriasis, systemic lupus erythematosis</td>
</tr>
<tr>
<td>TLR6</td>
<td>Bacterial cell wall components, modulin (phenol-soluble)</td>
<td>Cancer, type 2 diabetes, psoriasis, sepsis, leprosy, arthritis</td>
</tr>
<tr>
<td>TLR7</td>
<td>Self ssRNA, ssRNA, bropirimine, loxoribine, imidazoquinoline</td>
<td>Cancer, systemic lupus erythematosis, rheumatoid arthritis, arthritis</td>
</tr>
<tr>
<td>TLR8</td>
<td>Self ssRNA, small synthetic compounds, imidazoquinoline</td>
<td>Cancer, rheumatoid arthritis, viral keratitis, Crimean-Congo hemorrhagic fever</td>
</tr>
<tr>
<td>TLR9</td>
<td>Self dsDNA, unmethylated CpG DNA</td>
<td>Allergies, asthma, cancer, multiple sclerosis, systemic lupus erythematosis, Graves’ ophthalmopathy, Crimean-Congo hemorrhagic fever, arthritis</td>
</tr>
<tr>
<td>TLR10</td>
<td>Unknown</td>
<td>Cancer, asthma, nephropathy, viral keratitis</td>
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</tr>
<tr>
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<td>Glycolipids, triacyl lipopeptides, heat shock proteins, rare LPS species (Porphyromonas gingivalis), lipopeptides, lipoteichoic acid, meso-diaminopimelic acid, muramic acid, Neisseria gonorrhoeae, peptidoglycan, zymosan (Beta-glucan), bacterial flagellin, Yersinia virulence factors, CMV virions, saturated fatty acids</td>
<td>Cancer, type 2 diabetes, psoriasis, pre-eclampsia, Alzheimer’s, herpes simplex encephalitis, rheumatoid arthritis, acne vulgaris, acute rheumatic fever, asthma, atherosclerosis, chronic obstructive pulmonary disease, diabetic nephropathy, subnephrotic dermatis</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA (self and viral), poly I:C</td>
<td>Cancer, type 1 diabetes, rheumatoid arthritis, Hashimoto’s thyroiditis, virus-associated autoimmune disease</td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipo-polyaccharides, saturated free fatty acids, fibrinogen, fibrinectin, heat shock proteins, flavopiridol, Streptococcus pneumoniae pneumonia, heparin sulfate, hyaluronic acid, high mobility group box 1 protein, MMTV envelope proteins, nickel, paclitaxel, RSV fusion protein, respiratory syncytial virus coat protein, muramidase polymers, teichuronic acids, bacterial flagellin, surfactant protein A, beta-defensin 2</td>
<td>Cancer, type 2 diabetes, psoriasis, pre-eclampsia, rheumatoid arthritis, chronic obstructive pulmonary disease, diabetic nephropathy, subnephrotic dermatis</td>
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dsRNA = double-stranded RNA; CMV = cytomegalovirus; DAMPs = damage-associated molecular patterns; MMTV = mouse mammary tumor virus; PAMPs = pathogen-associated molecular patterns; poly I:C = polyinosinic-polycytidylic acid; RSV = respiratory syncytial virus; ssRNA = single-stranded RNA; TLR = toll-like receptor.

Toll-like Receptors in Autoimmune and Aberrant Inflammatory Responses

Chronic TLR activation and signaling in both immune and nonimmune cells by environmental antigens are now also thought to play critical roles in the induction of many chronic inflammatory diseases, the initiation and/or perpetuation of autoimmunity (loss of self-recognition), as well as contribute to onogenesis, tumor growth, and spread.\textsuperscript{28} Much of our recent research has focused on the roles of TLR3 and TLR2/TLR4 in the viral-induction of T1DM and high-fat diet (HFD) or free fatty acid (FFA) induction of obesity, insulin resistance (IR), T2DM, and, more recently, NAFLD, respectively. These three TLRs and their signaling pathways are depicted in Figure 1. Once TLRs bind to their respective ligands, they dimerize and intracellular adaptor molecules regulate their downstream signaling. The primary effector molecules of TLR2 and TLR4 are the transcription factors: nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and interferon regulatory factor (IRF)-5 while for TLR3 they are NFκB and IRF3. These transcription factors then enter the cell nucleus and activate...
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US ENDOCRINOLGY

However, viruses are suspected as the principle environmental trigger in cases, which are more fulminant in onset, is unknown (T1DM-B); β predominately T-cell-mediated, gradual (months to years) autoimmune responses, as well as upregulation of MHC genes responsible for the production of both forms. The strongest genetic link to T1DM-A are the class II HLA genes that control antigen presentation within the innate and acquired immune systems, while the genes responsible for T1DM-B are poorly characterized (only single-gene polymorphism associations). The HLA haplotypes DR3,4DQ8 exhibit the highest predictive risk for T1DM-A, but the genetic component with this strongest haplotype is thought to be responsible for only 40–50 % of the total risk, leaving environmental induction or acceleration as a major factor again in both forms of T1DM.

While no single viral trigger for either form of T1DM has been clearly identified, the Coxsackie viruses (CBVs) are most closely associated with both forms, although influenza B, herpes simplex, and human herpes 6 virus have also been linked to both forms. In one study of children with new-onset T1DM, 39 % demonstrated a CBV-specific antibody. Some CBV strains have specific β-cell tropism, indicating that these cells may be specific targets for them, and a large research effort to develop a CBV-based vaccination for prevention of T1DM is underway. Finally, serum from patients with new-onset T1DM possess a subset of anti-CBV antibodies that recognize β-cell autoantigens and are able to induce β-cell apoptosis in a pancreatic beta cell line.

The Role of Viruses in the Pathogenesis of T1DM

T1DM results from β-cell destruction and the consequent loss of endogenous insulin secretion. There are recognized by TLR3 on the edosome, initiate their downstream signaling, which mediates both the innate and acquired immune response via upregulation of major histocompatibility (MHC) class I antigens initially followed by self-antigens. Anti-GAD = anti-glutamic acid decarboxylase; CBCL10 = chemokine (C-X-C motif) ligand 10; ICAG = -cell autoantibody; IL-1β = interleukin-1 beta; insulinoma antibody = IA-2AA; type 1 IFNs = type I interferons.

Figure 1: TLR2, TLR3, and TLR4 Signaling Pathways

TLR2 and TLR4 are surface receptors while TLR3 is located on the endosome. Once activated, they dimerize and the primary effector molecules of TLR2 and TLR4 are the transcription factors: nuclear factor kappab-light-chain-enhancer of activated B cells (NFkB) and interferon regulatory factor (IRF)-5, while NFkB and IRF-3 are activated by TLR3. NFkB, IL-1β, and IRF-3 are translocated to the nucleus and result in synthesis and release of their respective cytokines. AP-1 = activator protein 1; BCL10 = -cell lymphoma/leukemia 10; CXCL10 = chemokine (C-X-C motif) ligand 10; DDX3 = (Asp-Glu-Ala-Asp) Box Helicase 3, X-linked; ERK = extracellular-signal-regulated kinases; IKKα = inhibitor of nuclear factor kappa-B kinase subunit alpha; IKKβ = nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta; iKKβ = inhibitor of nuclear factor kappa-B kinase subunit beta; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; IRAK = interleukin-1 receptor-associated kinase; IRF-3IRF-3 binding protein-1; JNK = c-Jun N-terminal; MALT1 = mucosa-associated lymphoid tissue lymphoma translocation protein 1; MCP1 = monocyte chemotactic protein-1; MHC = major histocompatibility complex; MyD88 = myeloid differentiation primary response gene 88; NAP1 = nucleosome assembly protein 1-like 1; NEMO = inhibitor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta; NFκB = nuclear factor κ-light polypeptide gene enhancer in B-cells; NFκB = nuclear factor kappa-B; p38 = mitogen-activated protein kinase kinase; MyO88 = mitogen-activated protein kinase 7; TBK1 = TANK-binding kinase 1; TRAF = TNF-receptor-associated factor; TRAM = TRIF-related adaptor molecule; TRIF = TLR3 adaptor/inducing receptor-3.

The Role of Viruses in the Pathogenesis of T1DM

Both mouse and human pancreatic islets express multiple TLRs, including TLR2, TLR3, TLR4, and TLR9. TLR3 recognizes viral double-stranded RNA (dsRNA) while TLR9 recognizes self-DNA or unmethylated CpG DNA released from damaged β cells. Only TLR3 expression and signaling is upregulated in β cells by dsRNA, which is the intermediate nucleic acid recognized by TLR3 on the endosome, initiate their downstream signaling, which mediates both the innate and acquired immune response via upregulation of major histocompatibility (MHC) class I antigens initially followed by self-antigens. Anti-GAD = anti-glutamic acid decarboxylase; CBCL10 = chemokine (C-X-C motif) ligand 10; ICAG = -cell autoantibody; IL-1β = interleukin-1 beta; insulinoma antibody = IA-2AA; type 1 IFNs = type I interferons.

Figure 2: Role of Viruses and TLR3 in the Induction of Insulitis and T1DM

Viruses inject their nucleic acids into the cell to begin replication. There are recognized by TLR3 on the endosome, initiate their downstream signaling, which mediates both the innate and acquired immune response via upregulation of major histocompatibility (MHC) class I antigens initially followed by self-antigens. Anti-GAD = anti-glutamic acid decarboxylase; CBCL10 = chemokine (C-X-C motif) ligand 10; ICAG = -cell autoantibody; IL-1β = interleukin-1 beta; insulinoma antibody = IA-2AA; type 1 IFNs = type I interferons.
Diabetes

Pathogenesis of T2DM and NAFLD

T2DM and NAFLD are both chronic inflammatory diseases that are highly correlated with visceral obesity. Diets from industrialized countries include large volumes of processed foods, saturated fats, simple carbohydrates, and HFCS, which, coupled with decreased physical activity, is largely responsible for the epidemic of obesity and obesity-induced chronic inflammatory diseases including T2DM and NAFLD throughout the world. In the early stages of T2DM with weight gain and visceral obesity-induced IR, there is islet cell compensation with beta cell hyperplasia and hyperinsulinemia. Over time, however, this inevitably leads to progressive beta cell failure and insulin deficiency. The molecular pathogenesis of T2DM and NAFLD are depicted in Figure 3. Visceral adipocytes and associated macrophages are major contributors to these inflammatory processes via several signaling pathways. As visceral obesity increases (adipogenesis), both adipocytes and macrophages release increasing amounts of inflammatory adipokines/cytokines (TNF-α, IL-6, NF-kB, etc.)—a phenomenon that has been termed “low-grade sepsis” or “metabolic endotoxemia.” These adipokines/cytokines act locally to stimulate further adipogenesis as well as to downregulate the insulin receptor (IR) function, which induces IR in the adipocyte as well as multiple other insulin target tissues (liver, skeletal muscle). Once the storage capacity of visceral adipocytes is saturated, endogenous FFAs and triglycerides (TGs) are released into the portal and peripheral circulation and exogenous dietary FFAs and TGs can no longer be cleared from the blood following meals. Excess circulating FFAs and TGs are subsequently ectopically deposited in insulin target tissues, where they trigger the local release of the same inflammatory cytokines and chemokines (lipotoxicity), further exacerbating IR. The ectopic fat deposition in skeletal muscle results in the loss of mitochondrial function and consequent decreased oxidative phosphorylation, which also contributes to IR and interferes with weight loss. The ectopic fat deposited in the pancreas also induces β-cell autophagy contributing to accelerated β-cell death and ultimately insulin deficiency.

Role of the Carcinoembryonic Antigen-related Cell Adhesion Molecule 1 in the Pathogenesis of T2DM and NAFLD

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a functional substrate of the IR in the liver and normally functions to promote insulin endocytosis and clearance from the circulation, occurring simultaneously with acquisition of visceral obesity and development of hyperinsulinemia from the pancreas. In the liver, which is the major regulator of glucose levels via hepatic storage (glycogen) or release (gluconeogenesis), there is also a major downregulation of CEACAM1. Thus, downregulation of CEACAM1 contributes further to IR and hyperinsulinemia, as well as NAFLD. Proof for the role of CEACAM1 in the pathogenesis of both T2DM and NAFLD was demonstrated in mouse studies using both a general CEACAM1 knockout and selective hepatic CEACAM1 knockout where the animals developed visceral obesity, IR, and steatosis. Thus, ectopic fat deposition in the liver from excess portal (visceral) FFAs and TGs (lipotoxicity) and local CEACAM1 downregulation perpetuate a vicious cycle of inflammation, IR, and steatosis.

The Role of TLR2/TLR4 Signaling in T2DM and NAFLD

Bacterial glycolipids and triacyl lipopeptides are the principle ligands for TLR2 while bacterial LPS is the principle exogenous ligand for TLR4. Their signaling pathways are demonstrated in Figure 1. Exogenous and endogenous FFAs (such as palmitate) induce pathologic TLR4 signaling in...
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adipocytes, resulting in the release of TNF-α and IL-6, and the induction of IR. There is now increasing evidence that the pathologic expression of TLR2 and TLR4 in other insulin-sensitive tissues, including skeletal muscle, islets, and liver, are a major source of pro-inflammatory adipokine/cytokine (TNF-α, IL-6, NF-κB, etc.) production that leads to the "inflammatory storm" seen in T2DM and NAFLD. These same processes also contribute to as much as 40% of the total risk for the chronic inflammation-induced, and obesity-associated malignancies (esophagus, pancreas, liver, colon, gall bladder, endometrium, breast, kidney, and thyroid).

Gut Flora, TLRs, and Chronic Inflammatory Diseases

Many interesting studies linking changes in intestinal (gut) flora, TLR function, and chronic diseases such as inflammatory bowel disease but also including T1DM, T2DM, and NAFLD, have been reported recently in the literature. These studies suggest that different species of bacteria may contribute to the pathogenesis and/or protection from many diseases through activation and/or suppression of different TLR functions in gut epithelial cells and immune cells. Many early investigators suggested that the excessive TLR expression seen in obesity/T2DM was the result of gut bacteria-derived LPS and demonstrated that serum and tissue levels of LPS are higher in obesity/T2DM. Although fascinating in nature, a discussion of the gut microbiome, their linkage to TLR function, and to these diseases is beyond the scope of this discussion.

TLR as Therapeutic Targets

As soon as the functional role of TLRs were appreciated, they became potential therapeutic targets for various infectious diseases, allergic, immunologic and inflammatory diseases, sepsis, as well as the similar signaling pathways associated with cancer development and spread. The two principle sites for TLR targeting have been at their ligand binding sites or through inhibition of their downstream signaling pathways. Numerous strategies have been developed ranging from gene deletion to monoclonal antibodies. TLR-specific agonists and antagonists as well as inhibitors of the various TLR adaptor and regulatory molecules have also been described. A review of the current clinical trials using TLR-directed therapies highlighted at least eight compounds in 2014, wherein some used TLR3 and TLR9 agonists as vaccine adjuvants in HIV infections or chronic fatigue syndrome, while others used TLR7, TLR8, and TLR9 antagonists in various autoimmune diseases, and yet additional groups used TLR4 antagonists in sepsis trials.

Novel Inhibitor of Pathologic TLR Expression and Signaling in T1DM, T2DM, and NAFLD

C10 is a derivative of the anti-thyroidal medication methimazole. Although it does not block thyroxine synthesis it does possess unique anti-inflammatory properties, many of which are mediated through suppression of TLR signaling pathways in both immune and nonimmune cells. C10 was initially described when it was demonstrated that human thyrocytes from patients with Hashimoto’s thyroiditis expressed a functional TLR3. Moreover, TLR3 overexpression in these thyrocytes could be stimulated by viral infection (dsRNA), and reversed by C10 treatment. This suggested that C10 might possess both immunoregulatory and anti-tumor properties especially given the role of chronic inflammation in the transformation of Hashimoto’s thyroiditis into papillary thyroid cancer, as well as the impact of the immune response on survival in multiple cancers. C10 has now been shown to block TLR signaling in a variety of immunologic diseases as well as the basal expression of TLR3 and TLR4 in multiple cancers, suppressing tumor growth and spread in tissue cultures and tumor implants. Finally, in pancreatic cancer, C10 suppresses tumor growth and spread through the inhibition of TLR3-induced IRF3 dimerization, translocation to the nucleus, and gene transcription.

Potential Use of C10 in T1DM

We have now demonstrated that C10 prevents direct dsRNA poly I:C-induced cytotoxicity (apoptosis) in two β cell culture lines (TC-6 and NIT-1) through inhibition of poly I:C-induced TLR3 signaling and the consequent inhibition of many of the cytokines and chemokines associated with T1DM including: CXCL10, IFN-β, TNF-α, and MHC class I gene expression in vitro. Pathologic IRF3 signaling via TLRs has previously been linked to both forms of diabetes. Next, we demonstrated that C10 protects 8 week-old NOD mice from CVB4 acceleration of diabetes, which usually occurs at 2 weeks post-infection. We also demonstrated that 8-week-old TLR3 knockout (TLR3/-) NOD mice, but not wild-type NOD mice, infected with CVB4 were also protected from the CVB4-induced insulitis and onset of diabetes at two
Figure 5: Effect of C10 on Adipogenesis (A) and Steatosis (B)

A: The differentiation of 3T3L1 pre-adipocytes into adipocytes was blocked by C10 treatment; B: High-fat diet-fed BL6 mice exhibit profound steatosis at 16 weeks, which is significantly inhibited by C10

weeks post-infection, confirming that this was a TLR3-mediated process. These findings are summarized in Figure 4. We suspect that activation of TLR3 signaling in β cells occurs through stimulation of the programmed cell death-1 ligand 1 (PD-L1) or changes in T-regulatory cell (CD4+CD25+Foxp3+) or effector T-cell (CD8) ratios in islets during early development. Thus we are currently looking at the effects of C10 on TLR3 (+/+ and TLR3 (-/-) NOD mice to confirm this. We are also conducting studies to determine if C10 could preserve β cell function and insulin-secretory capacity following the initial onset of CVB4-induced hyperglycemia.

Potential Use of C10 in T2DM

The observations that C10 (1) inhibits FFA (palmitate) induction of TLR signaling in 3T3 L1 pre-adipocytes; (2) inhibits palmitate-induced TLR4 expression and inflammatory signaling (IL-6 and iNos) in 3T3L1 adipocytes; (3) inhibits LPS-stimulated RAW2647 macrophages in culture; (4) blocks palmitate-induced Socs-3 upregulation and IRS-1 serine 307 phosphorylation, which mediate IR in 3T3L1 cells; and (5) prevents 3T3L1 cells from differentiating into adipocytes (Figure 5A [unpublished data]), suggested that it might be effective in reducing obesity-induced IR. To this end, we subsequently demonstrated that C10 treatment reversed IR, improved glucose intolerance wherein we observed improved area under the curve (AUC) in glucose tolerance tests (GTTs) as well as reduced elevated serum insulin, c-peptide, and leptin levels in high-fat diet-fed BL6 male mice, and if C10 is administered at the onset of a high-fat diet feeding (prevention study), animals gained less weight and it delayed the onset of T2DM in these mice (manuscript in preparation).

Potential Use of C10 in NAFLD

Anecdotally while conducting the T2DM prevention study using C10 in the high-fat diet-fed BL6 male mouse model of T2DM, we noted at necropsy that the C10-treated animals had normal-appearing livers while sham-vehicle-injected animals exhibited enlarged, pale livers, which was consequently found histologically to be steatosis resulting from the high fat feeding (see Figure 5B). We are currently studying C10 and other small-molecule TLR inhibitors that we have developed, which also prevent high-fat diet-induced steatosis (unpublished data) in both high-fat diet and high-fructose diet prevention studies, to confirm that these compounds do indeed possess potential therapeutic action in NAFLD.

Conclusions

T1DM, T2DM, and NAFLD are all inflammatory diseases in which TLRs mediate important inflammatory pathways. We have developed a small molecule, prototypical drug (C10), which significantly inhibits many TLR-dependent inflammatory pathways and prevents viral-induction of T1DM in the NOD mouse model, and high-fat diet-induced visceral obesity, IR, and NAFLD in BL6 male mice fed a high-fat diet. Moreover, we have also developed an additional class of small molecule inhibitors of TLR signaling that may be more potent and have lower toxicities than C10, which we are currently testing for efficacy in preclinical animal models.
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