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Loss of toll-like receptor 3 function improves glucose tolerance and reduces liver steatosis in obese mice

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ABSTRACT

Objective. Emerging evidence suggests a link between innate immunity and development of type 2 diabetes mellitus (T2D); however, the molecular mechanisms linking them are not fully understood. Toll-like Receptor 3 (TLR3) is a pathogen pattern recognition receptor that recognizes the double-stranded RNA of microbial or mammalian origin and contributes to immune responses in the context of infections and chronic inflammation. The objective of this study was to determine whether TLR3 activity impacts insulin sensitivity and lipid metabolism.

Materials and Methods. Wild type (WT) and TLR3 knock-out ($TLR3^{-/-}$) mice were fed a high fat diet (HFD) and submitted to glucose tolerance tests (GTTs) over a period of 33 weeks. In another study, the same group of mice was treated with a neutralizing monoclonal antibody (mAb) against mouse TLR3.

Results. $TLR3^{-/-}$ mice fed an HFD developed obesity, although they exhibited improved glucose tolerance and lipid profiles compared with WT obese mice. In addition, the increase in liver weight and lipid content normally observed in WT mice on an HFD was significantly ameliorated in $TLR3^{-/-}$ mice. These changes were accompanied by up-regulation of genes involved in cholesterol efflux such as PPARδ, LXRα, and LXRα-targeting genes and down-regulation of pro-inflammatory cytokine and chemokine genes in obese $TLR3^{-/-}$ mice. Furthermore, global gene expression profiling in liver demonstrated TLR3-specific changes in both lipid biosynthesis and innate immune response pathways.

Abbreviations: EMT, epithelial–mesenchymal transition; FDR, false discovery rate; FFA, free fatty acids; G6P, glucose-6-phosphotase; GcK, glucokinase; GO, gene ontology; GTT, glucose tolerance test; HDL-C, high-density lipoprotein cholesterol; HFD, high fat diet; HOMA, homeostasis model assessment; IKK-B, inhibitor of nuclear factor kappa-B kinase subunit beta; IL-6, interleukin 6; ipGTT, intraperitoneal glucose tolerance test; IR, insulin resistance; IRF3, interferon regulatory factor 3; LDL-C, low-density lipoprotein cholesterol; LXR, liver X receptor; LXRα, liver X receptor alpha; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; PEPCK, phosphoenolpyruvate carboxykinase; PPARδ, peroxisome proliferator-activated receptor delta; PPARγ, peroxisome proliferator-activated receptor gamma; SREBP1, Sterol Regulatory Element Binding Protein 1A; $t_{1/2}$, half-life; T2D, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; TLR3, toll-like receptor 3; $TLR3^{-/-}$, TLR3 knock-out; TNFα, tumor necrosis factor alpha; WT, wild type.

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Conclusions. TLR3 affects glucose and lipid metabolism as well as inflammatory mediators, and findings in this study reveal a new role for TLR3 in metabolic homeostasis. This suggests antagonizing TLR3 may be a beneficial therapeutic approach for the treatment of metabolic diseases.

1. Introduction

It has been proposed that low grade sub-clinical inflammation associated with obesity contributes to the development of metabolic diseases such as insulin resistance, T2D, and CVD [1]. Development of these diseases is likely a consequence of genetic background and environmental factors such as aging, diet, sedentary lifestyle, stress, and infection. Among the environmental factors, significant attention has been focused on the fact that consumption of a western HFD is strongly linked with the development of hyperlipidemia, insulin resistance, T2D, and CVD [2]. It is also clear that increased amount of fat mass (obesity) is associated with excessive circulating levels of FFAs [3], adipokines [4], and pro-inflammatory cytokines, including TNFα and IL-6. These can interfere with insulin signaling [5–7]. TNFα, IL-6, and fatty acids are elevated during the acute phase reaction mediated by activation of innate immunity, suggesting their potential involvement in metabolic homeostasis. Indeed, there is emerging evidence linking innate immune pathways with the modulation of metabolic homeostasis [8–11].

The TLR family is functionally conserved from fly to man and has a pivotal role in innate immunity. To date, 13 members of the TLR family have been identified in mammals; these recognize a diverse range of ligands, including microbial-derived antigens, as well as endogenous factors such as those released as a result of cell stress and damage. The interaction of TLRs with their specific ligands triggers cascades of signaling events that lead to activation of transcription factors such as NF-κB and IRF-3 [10]; these, in turn, result in expression of cytokine and chemokine genes and downstream inflammatory processes, events that can contribute to the pathogenesis of several diseases. For example, TLR4 has been implicated in the pathogenesis of cardiovascular and metabolic diseases [9,12,13].

Among TLRs, TLR3 activation has been implicated in autoimmune and inflammatory diseases including Hashimoto's thyroiditis [14] and type 1 diabetes [15,16]. For example, the potential role of TLR3 signaling in atherosclerosis was suggested by studies that demonstrated the impact of TLR3 activation on cholesterol metabolism by inhibition of LXR signaling pathways [9]. Additionally, several hepatic cell types (i.e., Kupffer cells, biliary epithelial cells, and hepatocytes) express TLR3 and respond to the synthetic TLR3 ligand poly(I:C) [17]. However, it remains unclear whether TLR3 signaling *in vivo* can impact the development of metabolic diseases such as obesity, insulin resistance, and T2D. To better understand the physiological role of TLR3 in the development of obesity and insulin resistance, we characterized TLR3$^{-/-}$ mice fed an HFD [18]. Our findings indicate that TLR3 deficiency did not protect mice from HFD-induced weight gain, although it did protect them from glucose intolerance, as shown by improved insulin sensitivity. WT obese mice treated with a neutralizing anti-TLR3 mAb also showed improved insulin sensitivity. In addition, lipid and cholesterol metabolism was markedly improved in TLR3$^{-/-}$ animals, suggesting a role for TLR3 in the regulation of lipid and cholesterol homeostasis in response to a prolonged HFD.

2. Materials and methods

2.1. Mice and diet

TLR3$^{-/-}$ mice with a C57BL/6 background were obtained from Dr. Richard A. Flavell [18]. Both TLR3$^{-/-}$ and WT control mice (C57BL/6) were fed either normal chow or an HFD (Purina TestDiet #58126) consisting of 60.9% kcal fat and 20.8% kcal carbohydrates. Mice were maintained on a 12:12-h light–dark cycle with water and food ad libitum. All studies were conducted in accordance with the guidelines set forth by the Animal Research Committee of Janssen Pharmaceutical R&D.

2.2. Study design

The weight of each mouse was measured weekly and the data presented as means ± SD. During the study, GTT was performed by i.p. administration of glucose at 1.0 mg/g body weight every 2 weeks after an overnight fast. In addition, plasma insulin and selected adipokines/cytokines were measured following an overnight fast. At necropsy, liver samples were obtained for RNA isolation and histological analysis.

WT and TLR3$^{-/-}$ animals fed an HFD for 16 weeks were treated with a neutralizing mAb against mouse TLR3 (CNTO5429). CNTO5429 is a mouse IgG1k mAb developed by Janssen Pharmaceutical R&D, which binds mouse TLR3 and neutralizes mouse TLR3 signaling *in vitro* (IC_{50} in a NF-κB reporter gene assay is 0.45 μg/mL or 3 nM) [19]. The $t_{1/2}$ of CNTO5429 following sc dosing in C57BL/6 and TLR3$^{-/-}$ mice was 2.5 and 11.5 days, respectively (data not shown), suggesting a receptor-dependent uptake of the mAb from systemic circulation. Mice were dosed i.p. with either 5 or 20 mg/kg over the study period of 7 weeks (2 doses during the first week and 1 dose every week thereafter for a total of 6 weeks).

2.3. Blood chemistry

Tail vein blood glucose levels were determined using a handheld glucometer (OneTouch Basic, LifeScan, Skilman, NJ). Plasma insulin levels were assessed from plasma using the Ultra-Sensitive ELISA Assay Kit (Crystal Chem, Downers Grove, IL). Plasma TNFα levels were determined using Lincoplex kits (#madpk-71k) from Linco Research, St. Charles, MO. Plasma levels of TC, HDL-C, LDL-C, and TG were measured