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A role for TLR10 in obesity and adipose tissue morphology

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ABSTRACT

Toll like receptors (TLRs) are expressed in adipose tissue and promote adipose tissue inflammation during obesity. Recently, anti-inflammatory properties have been attributed to TLR10 in myeloid cells, the only member of the TLR family with inhibitory activity. In order to assess whether TLR10-induced inhibition of inflammation may be protective during the development of obesity and metabolic abnormalities we used transgenic human *TLR10* mice (hTLR10tg) and wild type (WT) controls on a C57B6J background. HFD-feeding enhanced *TLR10* expression in the adipose tissue, and HFD-fed hTLR10tg mice displayed reduced adipocyte size, adipose tissue weight, and a trend toward lower plasma insulin levels compared to WT mice.

In humans, obese individuals with polymorphisms in the *TLR10* gene displayed reduced macrophage infiltration in the adipose tissue accompanied by a trend to lower leptin levels and higher adiponectin levels in plasma. In healthy individuals with the same polymorphisms in the *TLR10* gene we did not observe any difference in plasma concentrations of leptin and adiponectin.

We conclude that TLR10 impacts adipose tissue morphology in obesity. Larger studies in humans are warranted to assess its potential value as therapeutic target in metabolic syndrome and type 2 diabetes.

1. Introduction

The prevalence of obesity is rising worldwide promoting the development of the metabolic syndrome and closely related metabolic disturbances such as insulin resistance and type 2 diabetes [1]. Adipose tissue from obese individuals is characterized by an increased presence of pro-inflammatory macrophages [2]. Various animal studies have shown similar outcomes and subsequently demonstrated that the pro-inflammatory macrophage phenotype contributes to the development of inflammation that interferes with insulin signaling pathways. Ultimately, this pro-inflammatory trait leads to the development of insulin resistance during obesity [1,3].

Toll-like receptors (TLRs) have been shown to contribute to the development of obesity-induced inflammation [4]. As innate immunity pattern recognition receptors (PRR), TLRs sense pathogen-components to induce an inflammatory response. Moreover, recently endogenous activators of TLRs have been identified including free fatty acids [5]. In the adipose tissue TLRs are expressed on both macrophages and adipocytes and were described to be involved in the development of metabolic abnormalities including insulin resistance [4,6].

Numerous in vitro studies have shown that TLR2 and TLR4 activation promotes insulin resistance by inducing activation of JNK, p38 and IKK kinases that inhibit the phosphorylation of the insulin receptor substrate thus disturbing insulin signaling [4,6–8]. In support of these results, several studies in murine experimental models revealed that absence of TLR2 or TLR4 protects against the development of obesity-induced insulin resistance [9]. In obese individuals, high levels of free fatty acids are thought to either directly or indirectly drive the TLR4-signaling cascade in adipose tissue resulting in pro-inflammatory cytokine secretion and subsequent deterioration in insulin signalling pathways [10].

Recently, TLR10, a member of the TLR family receiving little attention so far, has been described to be the only TLR family member with anti-inflammatory properties in human myeloid cells. Blocking

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TLR10 in monocytes was proven to increase pro-inflammatory cytokine production upon stimulation with various stimuli [11]. Moreover, single nucleotide polymorphisms (SNPs) in the *TLR10* gene correlated with altered levels of cytokine production [11]. Various mechanisms of action may explain the anti-inflammatory effects of TLR10. First, TLR10 can form dimmers with other TLRs such as TLR1, TLR2 and TLR6 thereby blocking their signaling upon ligand binding. Second, TLR10 has been shown to promote the production of the anti-inflammatory cytokine IL-1Ra [11]. Moreover, a recent study showed that TLR10 has the capacity to block signals from other TLRs by suppressing both MyD88- and TRIF-dependent signaling [12].

In order to investigate whether TLR10 has anti-inflammatory properties in adipose tissue and to decipher the role of this receptor during the development of obesity and associated metabolic abnormalities, we used various approaches involving both animal and human studies. Firstly, we investigated the impact of a high-fat diet (HFD) versus low-fat diet (LFD) in *TLR10* transgenic mice. Secondly, we evaluate metabolic effects of polymorphisms in the *TLR10* gene in two human cohorts including both obese individuals and healthy controls.

Our results revealed upregulation of h*TLR10* in adipose tissue of mice during HFD-feeding. Moreover, despite similar weight gain upon LFD- or HFD-feeding, hTLR10tg mice had reduced adipose tissue weight, smaller adipocytes, and a trend towards less crown-like structures (CLSs) and lower circulating insulin levels than their wild type counterparts. In obese individuals carrying SNPs in the *TLR10* gene we observed reduced macrophage numbers in the adipose tissue and a trend to lower leptin plasma levels and higher adiponectin plasma levels whereas no differences in adipokine levels were observed in healthy controls carrying the same SNPs in *TLR10*.

2. Materials and methods

2.1. Human cohorts

302 obese individuals (IN-CONTROL study of the Cardiovascular research Netherlands Project) and more than 500 individuals (500 Functional Genomics Project) from the Human Functional Genomics project (HGFP) were genotyped as described before [13]. Of all individuals of the IN-CONTROL study and a total of 427 individuals of the 500 Functional Genomics Project, BMI and adipokines levels (leptin and adiponectin) could be measured and compared between individuals carrying a SNP and individuals with wild type (WT) alleles of the *TLR10* gene. Leptin and adiponectin concentrations in plasma were measured by ELISA according to manufacturer's protocol (R&D, The Netherlands). In addition, subcutaneous adipose tissue was obtained from 265 out of 302 obese individuals by performing needle biopsy after informed consent was obtained. The tissue was formalin fixed and paraffin-embedded and cross-sections (5μm) were stained by using the DAB (3′3 diaminobenzidine) method and anti-human CD68 antibody (Bio Rad, Veenendaal, The Netherands). Adipocyte size and the number of CLSs could be determined for 265 individuals. The HGFP was approved by the Ethical Committee of Radboud University Medical Center, Nijmegen (no. 42561.091.12). Experiments were conducted according to the principles expressed in the Declaration of Helsinki. Samples of venous blood were drawn after informed consent was obtained. For more information see http://www.humanfunctionalgenomics.org/site/.

2.2. Animal study

Because mice lack a functional *TLR10* gene, a constitutive human *TLR10* knock-in mouse was generated by Taconic Artemis using targeted transgenesis. Using recombination-mediated cassette exchange (RMCE), a CAG promoter cassette, the human *TLR10* open reading frame (ORF), and the hGH polyadenylation signal and an additional polyadenylation signal, were inserted into the ROSA26 locus. This obtained RMCE vector was transfected into the TaconicArtemis-C57BL/6 ES cell line. Using positive Neomycin selection, recombinant clones were selected. Positively selected blastocytes were transferred into embryos through which chimeric mice were attained. Using a Caliper LabChip GX device, sample analysis has been performed. Animals were fully back-crossed to a C57/Black6 background before they were used for experiments. Genotyping of the mice was performed using PCR and 1.5% agarose gel electrophoresis with the following primers: wild type forward 5′CTCTTCCCTCGTGATCTGCAACTCC; wild type reverse: 5′ CATGTCTTTAATCTACCTCGATGG; *TLR10* cond-forward: 5′GACAGCA GAGGGTGATGCTC; *TLR10* cond-reverse: 5′CTTCCTCACAGATAGGCA TGG; positive control forward: 5′GAGACTCTGGCTACTCATCC; positive control reverse: 5′CCTTCAGCAAGAGCTGGGGAC. The PCR conditions were: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s, followed by 72 °C for 10 min.

Human TLR10 transgenic (hTLR10tg) and C57BL/6j (WT) male mice aged between 12 and 16 weeks were given a low fat diet (LFD) or a high fat diet (HFD) with 10%, respectively 45%, of energy derived from fat [14] for 16 weeks (n = 9 for the HFD-fed mice, n = 10 for the LFD-fed mice). Bodyweight and food intake was measured weekly. Glycaemic control was assessed by an oral glucose tolerance test (oGTT). Mice were fasted overnight after which glucose was administered (2g/kg) followed by measurements of circulating glucose at 20, 40, 60, 90 and 120 min after glucose administration. Blood glucose concentration was measured with an Accu-chek glucosemeter (Roche Diagnostics, Almere, The Netherlands). The study was approved by the animal experimental committee in Nijmegen, The Netherlands, and all experiments were performed according to approved guidelines (Animal Research Facility - Radboudumc).

2.3. Plasma measurements

Triglycerides (TG) were determined using commercially available enzymatic kits from Roche Molecular Biochemicals (Indianapolis, IN). Free fatty acids (FFAs) were measured using the NEFA-C kit from Wako Diagnostics (Instruchemie, Delfzijl, The Netherlands). Plasma insulin concentrations were measured by ELISA according to the manufacturer's instructions (ultra sensitive mouse insulin ELISA kit, Crystal Chem Inc., IL, USA). Glucose (Liquicolor, Human GmbH, Wiesbaden, Germany) was measured enzymatically following manufacturer's protocols. Leptin, adiponectin and IL-1Ra were determined by ELISA according to manufacturer's protocol (R&D Systems, The Netherlands). Serum amyloid A (SAA) was measured with an ELISA assay (Tridelta Development Ltd., Maynooth, Ireland).

2.4. Liver, pancreas and adipose tissue histology

Liver and gonadal adipose tissue (gWAT) were formalin-fixed and paraffin-embedded, and cross-sections (5 μm) were stained by the DAB (3′3 diaminobenzidine) method using an anti-F4/80 antibody (AbD Serotec). Inflammation was assessed by determining the number of crown-like structures (CLSs) in four non-overlapping fields (at 20× magnification) using software as previously described [15]. Pancreatic tissue was stained with the DAB (3′3 diaminobenzidine) method using an anti-insulin antibody from Santa Cruz biotechnology. The Langerhans islets surface was determined using the software Image J (https://imagej.net/Welcome).

2.5. RNA isolation and gene expression analysis

After dissection, tissues were snap frozen using liquid nitrogen and stored at −80 °C before further processing. RNA was isolated using TRIzol reagent (Life Technologies Europe BV, Bleiswijk, The Netherlands) and quantity was measured with a Nano Drop Spectrophotometer (Nanodrop technologies, Montchanin, DE, USA). For gene expression analysis, cDNA was generated using reverse transcription with iScript (Biorad) following manufacturer's instructions.