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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

TRIF Differentially Regulates Hepatic Steatosis and Inflammation/Fibrosis in Mice

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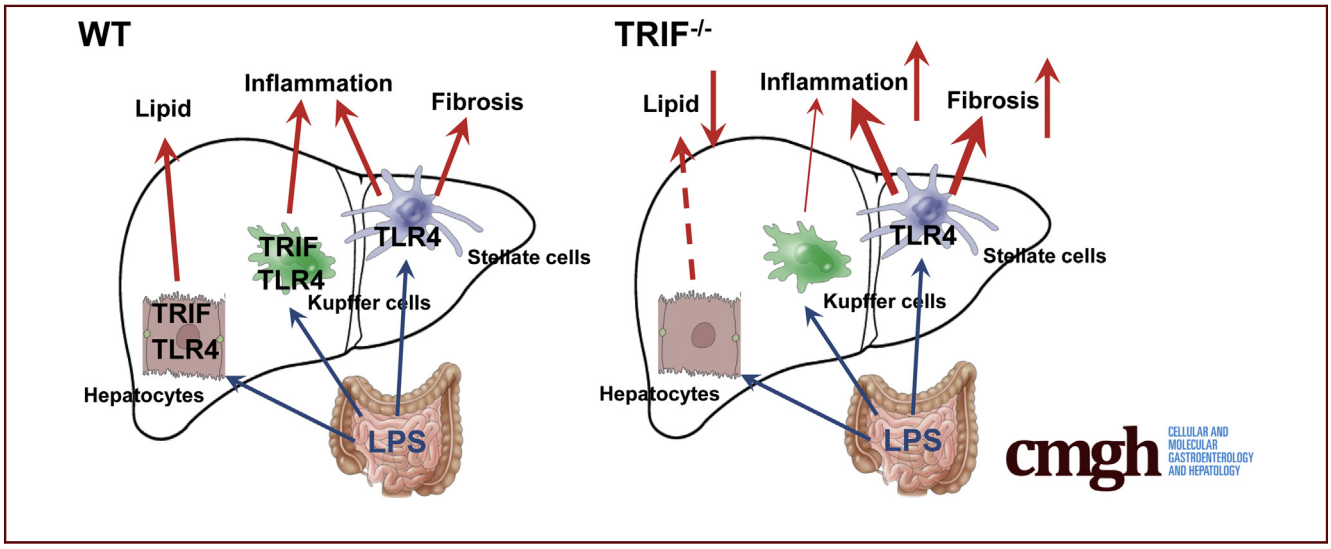

SUMMARY

Translocated intestine-derived lipopolysaccharide stimulates Toll-like receptor 4 expressed in different liver cells that have distinct roles in the development of nonalcoholic steatohepatitis. The Toll-like receptor 4–mediated TIR-domain containing adaptor-inducing interferon-β pathway promotes liver steatosis, but inhibits inflammation and fibrosis in nonalcoholic steatohepatitis.

BACKGROUND & AIMS: Toll-like receptor 4 (TLR4) signaling is activated through 2 adaptor proteins: MyD88 and TIR-domain containing adaptor-inducing interferon-β (TRIF). TLR4 and MyD88 are crucial in nonalcoholic steatohepatitis (NASH) and fibrosis. However, the role of TRIF in TLR4-mediated NASH and fibrosis has been elusive. This study investigated the differential roles of TRIF in hepatic steatosis and inflammation/fibrosis.

METHODS: A choline-deficient amino acid defined (CDAA) diet was used for the mouse NASH model. On this diet, the mice develop hepatic steatosis, inflammation, and fibrosis. TLR4 wild-type and TLR4$^{-/-}$ bone marrow chimeric mice and TRIF$^{-/-}$ mice were fed CDAA or a control diet for 22 weeks. Hepatic steatosis, inflammation, and fibrosis were examined.

RESULTS: In the CDAA diet–induced NASH, the mice with wild-type bone marrow had higher alanine aminotransferase and hepatic tumor necrosis factor levels than the mice with TLR4$^{-/-}$ bone marrow. The nonalcoholic fatty liver disease activity score showed that both wild-type and TLR4$^{-/-}$ bone marrow chimeras had reduced hepatic steatosis, and that both types of chimeras had similar levels of inflammation and hepatocyte ballooning to whole-body wild-type mice. Notably, wild-type recipients showed more liver fibrosis than TLR4$^{-/-}$ recipients. Although TRIF$^{-/-}$ mice showed reduced hepatic steatosis, these mice showed more liver injury, inflammation, and fibrosis than wild-type mice. TRIF$^{-/-}$ stellate cells and hepatocytes produced more C-X-C motif chemokine ligand 1 (CXCL1) and C-C motif chemokine ligand than wild-type cells in response to lipopolysaccharide. Consistently, TRIF$^{-/-}$ mice showed increased CXCL1 and CCL3 expression along with neutrophil and macrophage infiltration, which promotes liver inflammation and injury.

CONCLUSIONS: In TLR4-mediated NASH, different liver cells have distinct roles in hepatic steatosis, inflammation, and fibrosis. TRIF promotes hepatic steatosis but it inhibits injury,

inflammation, and fibrosis. *(Cell Mol Gastroenterol Hepatol 2017;3:469–483; http://dx.doi.org/10.1016/j.jcmgh.2016.12.004)*

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See editorial on page 299.

Fatty liver disease is the result of excessive fat accumulation in hepatocytes. Nonalcoholic fatty liver disease (NAFLD) is a manifestation of the metabolic syndrome, commonly associated with obesity and insulin resistance. The spectrum of NAFLD ranges from simple steatosis to steatosis accompanied by hepatocyte ballooning, inflammation, and fibrosis, referred to as nonalcoholic steatohepatitis (NASH).[1–4] NASH can progress to cirrhosis, which significantly increases the risk of hepatocellular carcinoma.[1–4] To date, there is no effective preventive or therapeutic agent for NASH and its associated fibrosis and hepatocellular carcinoma. Therefore, a better understanding of the underlying molecular mechanism is critical for the development of new and effective therapies.

Consumption of diets containing excessive fat and sugar is a risk factor for metabolic syndrome. These so-called Western-style diets may cause intestinal bacterial overgrowth as well as alter the composition of the intestinal microbiome. The altered intestinal microbiome may participate in endotoxin production, endogenous alcohol production, bile acid metabolism, and choline metabolism. Western-style diets form a gut microbiota that converts dietary choline into methylamines, reducing plasma levels of phosphatidylcholine. Because phosphatidylcholine is required for the secretion of very low density lipoprotein from hepatocytes, reduced choline levels can exacerbate fat accumulation in hepatocytes.[5]

Increased blood lipopolysaccharide (LPS) levels often are observed in patients with NASH.[5–7] Toll-like receptor 4 (TLR4) is a pattern-recognition receptor for LPS and induces activation of innate immune signaling through adaptor proteins MyD88 and TIR-domain containing adaptor-inducing interferon-β (TRIF).[8] Previous studies clearly have shown the critical roles of TLR4 and MyD88 in promoting NASH and its related fibrosis.[9–11] Interestingly, Interferon regulatory factor 3 (IRF3), downstream of the TRIF-dependent pathway, plays paradoxic roles between alcoholic liver disease and high-fat diet (HFD)-induced NAFLD. The TRIF-mediated IRF3 activation promotes alcohol-induced liver injury whereas IRF3 negatively regulates HFD-induced steatosis.[12,13] However, the precise roles of the TLR4–TRIF pathway in NASH and fibrosis remain elusive.

HFD feeding induces fatty liver, weight gain, and insulin resistance, but liver inflammation is very mild and fibrosis does not occur. In contrast, feeding of a methionine-choline–deficient diet, commonly used for NASH studies in rodents, induces liver injury and inflammation, but body weight is reduced and insulin resistance is not developed. Notably, rodents fed a choline-deficient amino acid defined (CDAA) diet evidently develop fibrosis along with hepatic steatosis, inflammation, weight gain, and mild insulin resistance.[10] Because we aimed to examine TLR4-mediated NASH fibrosis, this study used CDAA diet feeding to understand the mechanisms of the pathogenesis of NASH fibrosis. The present study investigated to determine the responsible cell types for TLR4-mediated NASH and fibrosis, and the role of TRIF in the development of NASH and its related fibrosis.

Materials and Methods

Mouse Colonies

Wild-type C57BL/6 mice and *Trif*$^{lps/lps}$ mice (hereafter referred to as TRIF$^{-/-}$ mice) were purchased from The Jackson Laboratory (Bar Harbor, MA).[14] TLR4$^{-/-}$ mice and MyD88$^{-/-}$ mice originally were developed in Shizuo Akira's laboratory and were back-crossed to C57BL/6 for more than 10 generations.[15] Male mice were divided into 2 groups at 8 weeks old: choline-supplemented L-amino acid–defined diet (catalog no. 518754; Dyets, Inc, Bethlehem, PA) and CDAA (catalog no. 518753; Dyets, Inc).[10] These diets were continued for 22 weeks. The mice received humane care based on the National Institutes of Health recommendations outlined in the Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the University of California San Diego and Cedars-Sinai Medical Center Institutional Animal Care and Use Committee.

Bone Marrow Transplantation

We performed bone marrow transplantation (BMT) experiments as previously described.[14] Briefly, bone marrow (BM) cells (1×10^7 cells) obtained from wild-type and TLR4$^{-/-}$ mice were transplanted through tail veins after the recipient mice were lethally irradiated (10 Gy). After 2 weeks of lethal irradiation followed by BMT, liposomal clodronate (200 μL intravenously) was injected to deplete liver resident macrophages, Kupffer cells, which accelerate hepatic macrophage turnover with BM cells. The mice were fed with CDAA and choline-supplemented amino acid–defined diet after 10 weeks of BMT. These diets were continued for 22 weeks. Some mice were transplanted with BM from β-actin promoter–driven green fluorescent protein (GFP) transgenic mice for assessing the efficiency of the engraftment of transplanted BM cells.

***Authors share co-first authorship.**

Abbreviations used in this paper: **ALT, alanine aminotransferase; BM, bone marrow; BMT, bone marrow transplantation; CDAA, choline-deficient amino acid defined; DGAT2, diacylglycerol acyltransferase 2; HFD, high-fat diet; HSC, hepatic stellate cell; IL, interleukin; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCR, polymerase chain reaction; α-SMA, α-smooth muscle actin; TLR4, Toll-like receptor 4; TNF, tumor necrosis factor.**

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