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Non-alcoholic steatohepatitis-induced fibrosis: Toll-like receptors, reactive oxygen species and Jun N-terminal kinase

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Abstract

Non-alcoholic steatohepatitis (NASH) represents the progression of hepatic steatosis to steatohepatitis, fibrosis and cirrhosis. Three signaling pathways have been associated with this progression; Toll-like receptors, reactive oxygen species and Jun N-terminal kinase. This review will describe how activation of these three pathways is required for development of fibrosis in murine models of NASH. The three pathways are related and synergistic through intracellular cross-talk. Disruption of any of these pathways may inhibit NASH-induced fibrosis and are potential targets for therapeutic intervention.

Keywords

fibrosis; Jun N-terminal kinase; liver; reactive oxygen species; Toll-like receptor

Non-Alcoholic Steatohepatitis (NASH) is a result of multiple environmental factors acting on the liver. Obesity induced by overnutrition is the most common environmental factor. Insulin resistance, which is closely associated with obesity, also contributes to steatosis with lipogenesis in the absence of proper glycolysis. Finally, recent evidence has demonstrated that the enteric microbiome contributes to both the metabolism and the inflammation in NASH. The steatosis that first defines non-alcoholic liver disease (NAFLD) may progress to steatohepatitis, fibrosis and cirrhosis. Three signaling pathways have been associated with this progression, which are Toll-like receptors (TLR), reactive oxygen species (ROS) and Jun N-terminal kinase (JNK).

The TLR, ROS and JNK are actually closely associated through intracellular cross-talk (see Fig. 1). The bacterial products that result from the enteric flora and increased intestinal permeability into the portal blood will activate the TLR. In turn, the intracellular signaling of the TLR will activate nuclear factor (NF)κB and JNK1. NFκB and JNK1 in turn will collaborate to induce chemokines and cytokines, which will produce both inflammation and fibrosis. Furthermore, TLR will activate NFκB which we have shown will decrease the expression of the transforming growth factor (TGF)-β pseudoreceptor Bambi, which in turn will upregulate TGF-β signaling, again leading to increased fibrosis. Through completely separate receptor families, angiotensin-2, leptin, platelet-derived growth factor (PDGF) and other agonists will activate their cognate receptors which are closely associated with the induction of the nicotinamide adenine dinucleotide phosphate oxidases (NOX). NOX in turn

will generate reactive oxidase species, which will lead to fibrosis directly, or will act to inhibit the map kinase phosphatase (MKP)-1 activity, which in turn will derepress JNK1 and further stimulate inflammation (see Fig. 1).

Our first study to demonstrate the importance of these pathways is the study that demonstrates that TLR9 mediates steatosis and fibrosis through interleukin (IL)-1 β in diet-induced steatohepatitis in mice.¹ TLR9 is activated by its ligand CpG DNA, which is found in the bacterial genome. This in turn activates MyD88, which leads to downstream activation of both an interferon pathway and a pathway activating NF- κ B and JNK.

The aim of our study was to investigate the role of TLR9 and its downstream target IL-1 β in the pathogenesis of steatohepatitis and subsequent liver fibrosis. Wild-type, TLR9 knockout, IL-1 receptor knockout, and MyD88 knockout mice were fed a choline-deficient amino acid-defined (CDAA) diet for 22 weeks and assessed for steatohepatitis, fibrosis and insulin resistance. Lipid accumulation and cell death were assessed in isolated hepatocytes. Kupffer cells and hepatic stellate cells were isolated to assess inflammatory and fibrogenic responses.

As expected, the CDAA diet induced NASH in wild-type mice, characterized by steatosis, inflammation, fibrosis and insulin resistance. On the other hand, TLR9 knockout mice showed less severe steatohepatitis and liver fibrosis than in wild-type mice. The reduced steatohepatitis in TLR9 knockout mice was associated with reduced IL-1 β levels but not with reduced levels of other inflammatory cytokines. Cell fractionation of the liver cells demonstrated that the Kupffer cells/macrophages were the major source of IL-1 β in this diet-induced model of NASH. Furthermore, activation of TLR9 by CpG DNA produced IL-1 β in cultured Kupffer cells. In turn, IL-1 β induced fibrogenic gene expression in primary cultures of hepatic stellate cells. Furthermore, IL-1 β increased lipid accumulation in cultured hepatocytes. Hepatocytes from CDAA diet fed mice were more susceptible to IL-1 β -induced cell death. When IL-1R1 knockout mice were fed the CDAA diet, these mice also had reduced steatohepatitis and fibrosis compared with wild-type mice. Finally, MyD88 knockout mice also had reduced steatohepatitis and fibrosis, demonstrating the relationship between IL-1R and MyD88. These results (see Fig. 2) demonstrate that TLR9 mediates IL-1 β production that in turn promotes steatohepatitis and fibrosis. In particular, TLR9 ligands, such as CpG DNA, activate TLR9 on Kupffer cells, which leads to the increased production of the inflammatory cytokine IL-1 β . IL-1 β in turn activates a fibrogenic cascade in hepatic stellate cells and leads to either lipid accumulation or apoptosis in hepatocytes. These combined results of insulin resistance, steatosis, hepatic injury, inflammation and fibrosis provide a potent mechanism by which TLR9 ligands are critical in NASH.

The next study demonstrates that NOX induces fibrosis, but not steatosis, in diet-induced steatohepatitis in mice.² The NOX family consists of six genes, all of which generate superoxide. NOX2 (gp91 PHOX) is a phagocytic form of NOX that is expressed in macrophages, including Kupffer cells, and is involved in bacterial killing. NOX1 is a non-phagocytic form of NOX that is expressed in smooth muscles cells, fibroblasts and as we recently demonstrated in hepatic stellate cells. This study investigated the role of NOX in ROS production with differential contribution of NOX from bone marrow (BM)-derived and non-BM-derived liver cells. Hepatic steatohepatitis and fibrosis was induced by the methionine choline-deficient (MCD) diet for 10 weeks in wild-type and p47phox knockout mice. P47phox is a component of multiple NOX proteins. The p47phox NOX knockout chimeric mice were generated by the combination of liposomal chlodronate injection, irradiation and BM transplantation of p47phox bone marrow into wild-type recipients and vice versa. Wild-type and p47phox knockout mice fed the MCD diet for 10 weeks showed comparable levels of steatosis as documented by staining and triglyceride content. However,

p47phox knockout mice had less fibrosis than wild-type mice as determined by Sirius red staining and expression of fibrogenic genes. Surprisingly, wild-type and p47phox knockout mice also had comparable levels of peroxidation, as measured by thiobarbituric acid. When wild-type or p47phox knockout hepatocytes were incubated with palmitic acid or oleic acid, they showed a similar increase in lipid deposition. Furthermore, wild-type and p47phox knockout hepatocytes showed similar increase in ROS production, as demonstrated by fluorescence. This study demonstrated that both phagocytic and non-phagocytic isoforms of NOX are involved in liver fibrosis. The non-phagocytic form of NOX expressed in hepatic stellate cells plays a major role in the contribution of liver fibrosis in mice. The p47 knockout mice treated with the MCD diet developed a similar level of steatosis but reduced fibrosis compared to wild-type mice. Thus, NOX is not involved in liver steatosis, but is critical for liver fibrosis.

Jun N-terminal kinase plays a pivotal role in the development of the metabolic syndrome, including NAFLD. However, the mechanism underlining the contribution of JNK to the progression from simple steatosis to steatohepatitis and liver fibrosis is unresolved. The aim of our study was to identify the distinctive roles of the JNK isoforms on the pathogenesis of steatohepatitis and subsequent liver fibrosis. Hepatic steatosis, inflammation and fibrosis were examined in wild-type, JNK1 knockout and JNK2 knockout mice fed a CDAA diet for 20 weeks. The functional contribution of JNK isoforms in Kupffer cells was assessed *in vitro* and *in vivo* using chimeric mice in which the hematopoietic compartment including Kupffer cells was replaced by wild-type, JNK1 knockout or JNK2 knockout cells.

Interestingly, wild-type, JNK1 knockout and JNK2 knockout mice all had the same severity of hepatic steatosis as documented by histology and hepatic triglyceride levels in this CDAA diet. However, JNK1 knockout mice had much less hepatic inflammation than wild-type or JNK2 knockout mice, as documented by histology and gene expression of inflammatory cytokines. Most importantly, JNK1 knockout mice had less liver fibrosis than wild-type or JNK2 knockout mice, as demonstrated by Sirius red staining and expression of fibrotic mRNA in the liver.³

Thus, JNK1 plays an important role in hepatic inflammation and liver fibrosis in this diet-induced model of NASH. Furthermore, hepatic inflammation, rather than steatosis, correlates with liver fibrosis. Fractionation of liver cells demonstrated that Kupffer cells/macrophages were the predominate source of all the inflammatory cytokines, as well as of TGF- β in the CDAA diet model. Examination of cultured Kupffer cells revealed that the JNK1 knockout Kupffer cells had a significant decrease in the production of the inflammatory cytokines in response to the bacterial product lipid polysaccharide. Thus, we assessed the role of Kupffer cells/macrophages in mediating the JNK1-required inflammation and fibrosis by generating chimeric mice and then feeding them the CDAA diet for 20 weeks. JNK1 deletion of the hematopoietic cells decreased CDAA diet-induced hepatic inflammation and fibrosis, as documented by histology and assessment of inflammatory and fibrogenic genes. Overall, this study demonstrates that in this model of NASH there is significantly less inflammation and fibrosis despite similar levels of hepatic steatosis in JNK1 knockout mice. Furthermore, the inflammation is mediated by the Kupffer cells/macrophages and JNK1 is required for Kupffer cell activation. Finally, JNK1 deletion in hematopoietic cells decreases the chronic inflammation and subsequent hepatic fibrosis in this model of NASH.³

REFERENCES

1. Miura K, Kodama Y, Inokuchi S, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1 β in mice. *Gastroenterology*. 2010; 139:323–334. e7. [PubMed: 20347818]

2. De Minicis S, Seki E, Paik YH, et al. Role and cellular source of nicotinamide adenine dinucleotide phosphate oxidase in hepatic fibrosis. *Hepatology*. 2010; 52:1420–1430. PMID: 2947612. [PubMed: 20690191]
3. Kodama Y, Kisseleva T, Iwaisako K, et al. c-Jun N-terminal kinase-1 from hematopoietic cells mediates progression from hepatic steatosis to steatohepatitis and fibrosis in mice. *Gastroenterology*. 2009; 137:1467–1477. e5. PMID: 2757473. [PubMed: 19549522]

Hepatic fibrosis: TLRs, NOX and JNK

Figure 1.

Hepatic fibrosis: Toll-like receptors (TLR), nicotinamide adenine dinucleotide phosphate oxidases (NOX) and Jun N-terminal kinase (JNK). MKP, map kinase phosphatase; NF-κb, nuclear factor κb; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF, transforming growth factor.

TLR9-mediates IL-1 β that promotes steatohepatitis and fibrosis

Figure 2. Toll-like receptor (TLR)9-mediates interleukin (IL)-1 β that promotes steatohepatitis and fibrosis. IL, interleukin; NF- κ B, nuclear factor κ B; TIMP, tissue inhibitor of metalloproteinases.