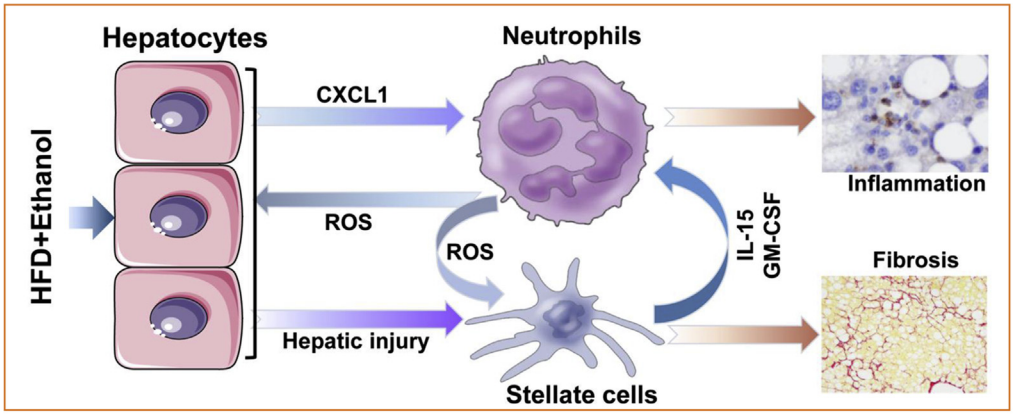
cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Neutrophil–Hepatic Stellate Cell Interactions Promote Fibrosis in Experimental Steatohepatitis

Zhou Zhou,[1,*] Ming-Jiang Xu,[1,*] Yan Cai,[1,*] Wei Wang,[1] Joy X. Jiang,[2] Zoltan V. Varga,[3] Dechun Feng,[1] Pal Pacher,[3] George Kunos,[4] Natalie J. Torok,[2] and Bin Gao[1]

[1]*Laboratory of Liver Diseases,* [3]*Laboratory of Cardiovascular Physiology and Tissue Injury,* [4]*Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland;* [2]*Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of California Davis Medical Center, Davis, California*



SUMMARY

The present study shows that activated hepatic stellate cells produce granulocyte-macrophage colony-stimulating factor and interleukin-15 to prolong the survival of neutrophils. This may serve as a feed-forward signaling loop that promotes steatohepatitis and liver fibrosis in high-fat diet plus binge ethanol–induced steatohepatitis.

BACKGROUND & AIMS: Hepatic infiltration of neutrophils is a hallmark of steatohepatitis; however, the role of neutrophils in the progression of steatohepatitis remains unknown.

METHODS: A clinically relevant mouse model of steatohepatitis induced by high-fat diet (HFD) plus binge ethanol feeding was used. Liver fibrosis was examined. *In vitro* cell culture was used to analyze the interaction of hepatic stellate cells (HSCs) and neutrophils.

RESULTS: HFD plus one binge ethanol (HFD+1B) feeding induced significant hepatic neutrophil infiltration, liver injury, and fibrosis. HFD plus multiple binges of ethanol (HFD+mB) caused more pronounced liver fibrosis. Microarray analyses showed that the most highly activated signaling pathway in this HFD+1B model was related to liver fibrosis and HSC activation. Blockade of chemokine (C-X-C motif) ligand 1 or intercellular adhesion molecule-1 expression reduced hepatic neutrophil infiltration and ameliorated liver injury and fibrosis. Disruption of the $p47^{phox}$ gene (also called *neutrophil cytosolic factor 1*), a critical component of reactive oxygen species producing nicotinamide adenine dinucleotide phosphate-oxidase in neutrophils, diminished HFD+1B–induced liver injury and fibrosis. Co-culture of HSCs with neutrophils, but not with neutrophil apoptotic bodies, induced HSC activation and prolonged neutrophil survival. Mechanistic studies showed that activated HSCs produce granulocyte-macrophage colony-stimulating factor and interleukin-15 to prolong the survival of neutrophils, which may serve as a positive forward loop to promote liver damage and fibrosis.

CONCLUSIONS: The current data from a mouse model of HFD plus binge ethanol feeding suggest that obesity and binge drinking synergize to promote liver fibrosis, which is partially mediated via the interaction of neutrophils and HSCs. Microarray data in this article have been uploaded to NCBI's Gene Expression Omnibus (GEO accession number: GSE98153). *(Cell Mol Gastroenterol Hepatol 2018;5:399–413; https://doi.org/10.1016/j.jcmgh.2018.01.003)*

Keywords: Alcohol; High-Fat Diet; Fatty Liver; Reactive Oxygen Species; Inflammation.

See editorial on page 424.

Liver disease is one of the leading global health problems, causing significant mortality worldwide. Although various risk factors have been discovered and the disease progression has been well characterized, the underlying mechanisms and therapeutic targets in various forms of liver disease are still elusive. Obesity[1,2] and alcohol drinking[3,4] are 2 well-known major risk factors for liver diseases, causing a similar spectrum of liver pathologies from simple fatty liver to more severe forms of liver injury, such as steatohepatitis, cirrhosis, and cancer. These 2 risk factors often co-exist and synergistically promote liver disease progression[5,6]; however, the underlying mechanisms remain obscure. Recently, we developed a clinically relevant mouse steatohepatitis model consisting of 3-month high-fat diet (HFD) feeding plus 1 binge of ethanol (HFD+1B).[7–9] Three-month HFD feeding alone caused obesity and severe fatty liver with a mild increase of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, but little or no hepatic neutrophil infiltration. Interestingly, acute gavage of a single dose of ethanol induced massive hepatic neutrophil infiltration and liver injury (increase of serum ALT and AST level) in 3-month HFD-fed mice,[7,8] providing a good model to study the role of neutrophils in liver injury.

Hepatic neutrophil infiltration is a critical pathologic feature in liver damage associated with both obesity and alcohol-related liver diseases.[10–12] Neutrophils are critical components of the innate immune system. Upon infection or tissue injury, neutrophils are rapidly recruited to the infected or injured areas to clear the bacteria or remove damaged cells. However, because of the lack of selectivity, neutrophils also may cause tissue damage along with their beneficial effects on host defense.[12–14] Hepatic neutrophil infiltration is a hallmark of alcoholic steatohepatitis in patients and also is observed in animal models of chronic plus binge ethanol feeding[15] and HFD+1B feeding.[7,8] The results of studies using these animal models suggest that neutrophil infiltration contributes to liver injury in steatohepatitis and have identified several mechanisms underlying hepatic neutrophil infiltration in these models.[7,8,15] For example, HFD+1B feeding highly up-regulated hepatic expression of chemokine (C-X-C motif) ligand 1 (CXCL1),[7] which is one of the most selective and powerful chemokines to attract neutrophils.[16] Inhibition of CXCL1 reduced hepatic neutrophil infiltration and ameliorated liver injury induced by HFD+1B feeding.[7] Moreover, up-regulation of hepatic *Cxcl1* gene expression in HFD-fed mice by acute ethanol binge is partially mediated via the inhibition of hepatic peroxisome proliferator-activated receptor γ, a negative regulator for *Cxcl1* gene expression.[8] Although HFD feeding and acute binge ethanol together are known to synergistically induce hepatic neutrophil infiltration and hepatocellular damage, whether this combined challenge also induces liver fibrosis remains unknown.

Liver fibrosis is the scarring process after liver injury, which may progress to cirrhosis and liver cancer.[17,18] Hepatic stellate cells (HSCs) are the major cell type responsible for liver fibrogenesis by producing matrix proteins during chronic liver injury.[17,19] In the quiescent state, HSCs store retinol inside their lipid droplets. During chronic liver inflammation and injury, HSCs become activated, lose lipid droplets, and differentiate into myofibroblasts, which express smooth muscle actin and various types of collagen proteins, resulting in extracellular matrix deposition and fibrosis. Over the past 4 decades, a large number of inflammatory mediators have been identified to control HSC activation.[18] For example, activation of natural killer cells has been well documented to inhibit liver fibrosis by directly killing activated HSCs and producing interferon-γ that induces HSC apoptosis and cell-cycle arrest[20]; whereas neutrophils likely promote liver fibrosis by inducing hepatocellular damage and HSC activation via the production of reactive oxygen species (ROS).[18] However, the exact functions of neutrophils in liver fibrogenesis in steatohepatitis have not been studied because of a lack of an animal model that recapitulates human steatohepatitis with significant neutrophil infiltration. The mouse model of steatohepatitis induced by HFD+1B feeding possesses several interesting features, including severe steatosis, a high increase of serum ALT and AST levels, and significant neutrophil infiltration in the liver.[7,8] In the current study, we performed microarray analyses using this model and found that the most robustly activated signaling pathway in the liver is related to liver fibrosis and HSC activation. Furthermore, we examined the role of neutrophils in liver fibrogenesis *in vivo* in this model, and also performed *in vitro* co-culture experiments to analyze the reciprocal interaction between neutrophils and HSCs. The data showed that apart from the already known effect of neutrophils on promoting HSC activation, activated HSCs enhanced the survival of neutrophils by producing granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)15, thereby exacerbating liver inflammation.

Materials and Methods

Mice

Male C57BL/6J, intercellular adhesion molecule-1 $(Icam\text{-}1)^{-/-}$, and $p47^{phox\text{-}/\text{-}}$ mice were purchased from the Jackson Laboratories (Bar Harbor, ME) and housed in a temperature-controlled, 12-hour light/12-hour dark room.

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

Abbreviations used in this paper: **ALT, alanine aminotransferase; AST, aspartate aminotransferase; cDNA, complementary DNA; *Csf*, colony-stimulating factor gene; CXCL1, chemokine (C-X-C motif) ligand 1; FBS, fetal bovine serum; 4-HNE, 4-hydroxynonenal; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; HFD, high-fat diet; HFD+mB, high-fat diet plus multiple binges; HFD+1B, high-fat diet feeding plus 1 binge of ethanol; HSC, hepatic stellate cell; ICAM-1, intercellular adhesion molecule-1; KO, knockout; MPO, myeloperoxidase; mRNA, messenger RNA; PCR, polymerase chain reaction; RT-PCR, reverse-transcription polymerase chain reaction; ROS, reactive oxygen species; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling; WT, wild-type.**

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2018.01.003